Antifungal Susceptibility Testing of Dermatophytes by ABDD and E-Test, a Comparative Study

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Abstract

Aim: The objective of this study was to isolate, identify, and explore the in-vitro antifungal susceptibility pattern of dermatophytes isolated from clinically suspected cases of dermatophytosis (tinea infections) attending the Dermatology Department at J.S.S Hospital. Methods: This study was conducted at JSS Medical College and Hospital from December 2016 to December 2017. Clinical samples (e.g., skin scrapings and hair stumps) were collected under aseptic precautions. The identification of dermatophytes was performed through microscopic examination using 10%, 20% & 40% potassium hydroxide (KOH) and culture on Sabouraud dextrose agar (SDA), SDAac, PDA and Dermatophyte test medium (DTM). All dermatophytes isolates were subjected to antifungal susceptibility testing using the agar-based disk diffusion (ABDD) and E-test method against Terbinafine, Itraconazole, Fluconazole, and Griseofulvin. Data were analyzed by using Chi square test. Results: A 100 samples were studied, 46% tinea corporis, 2% tinea cruris, 9% tinea pedis, 5% tinea faciei. The dermatophytes isolated were Trichophyton rubrum 11 (35%), Trichophyton mentagrophyte 8 (25%), Trichophyton tonsurans 5 (16%), Microsporum gypseum 3 (10%), Trichophyton verrucosum 2 (6%), Trichophyton violaceum 1 (3%) and Microsporum audouinii 1 (3%). Out of 31 dermatophytes 17 were sensitive to all four antifungal agents within the range of FLC (2 - 6 mcg/ml), ITR (0.125 - 2), TER (0.125 - 2), and GRI (0.125 - 2), 5 isolates were resistant in which 2 were resistant to FLC (64.256 mcg/ml), 2 isolates were resistant to TER 2 (32.38 mcg/ml), 1 isolate was resistant to both GRI and TER (16.32 mcg/ml) and 9 isolates were within the intermediate range. Conclusion: Every patient with a tinea infection should be properly studied for a mycological examination and should be treated accordingly. Dermatophyte test medium is more useful as an identification medium in the
isolation of dermatophytes. The ABDD method appears to be a simple, cost-effective, and promising method for the evaluation of antifungal susceptibility of dermatophytes. E-test method is the most sensitive method due to the fact that quantitative MICs can be obtained directly from the E-strip. However, the E-test method is expensive and difficult in defining the precise borders of the inhibition zones in dermatophytes.

Keywords
Dermatophytosis, Dermatophyte Test Medium, Disc Diffusion and E-Strips, Antifungal Agents

1. Introduction
Dermatophytes are a group of closely related filamentous fungi able to damage and utilize keratin found in the skin, hair and nails [1] [2]. These are classified into three genera: Microsporum, Trichophyton, and Epidermatophyton. Dermatophytoses is an infection produced by dermatophytic fungi in the keratinized tissues. Clinically, dermatophytoses can be classified depending on the site involved. These include Tinea capitis (scalp), Tinea corporis (non-hairy skin of the body), Tinea unguium (nail infection), tinea cruris (groin), Tinea pedis (athletes foot), and Tinea barabae (bearded areas of the face and neck) [1] [2]. World Health Organization (WHO) estimates dermatophytes affect about 25% of the world’s population. It is also estimated that 30% - 70% of adults are asymptomatic carriers of these pathogens, and that the incidence of this disease increases with age [1] [3]. The estimated life-time risk of acquiring dermatophytoses is between 10% - 20%. The global prevalence of dermatophytoses is estimated to be 20% [2] [4]. The prevalence of dermatophytes has increased tremendously in the last few decades due to various factors like climatic changes, socio-economical and occupational situations [5] [6].

The diagnosis of a dermatophytic infection is mostly done clinically, but often confused with other skin infections due to the topical application of steroid ointments and creams, leading to further misdiagnosis and mismanagement [4] [7]. Hence, there arises the need for the correct, efficient, and rapid laboratory diagnosis of dermatophytes [8]. Another important point to consider is that resistance to antifungals has started appearing in dermatophytes [9]. The establishment of a reference antifungal susceptibility testing method may allow the clinician to select the appropriate therapy for the treatment of infections caused by dermatophytic fungi [10] [11].

The antifungal susceptibility test of dermatophytes has been well-studied in some developed countries, but few data are available in tropical countries [12]. Resistance to antifungal agents is also on the increase in dermatophytes seeks to improve knowledge of the molecular identification and the antifungal susceptibility test of dermatophytes [13]. The agar-based disk diffusion (ABDD) suscep-
tibility method for dermatophytes is quick, easy, and inexpensive and does not require specialized equipment, making it a good option. The E-test results correlate well with those of the disk diffusion method [14] [15]. Unlike disk diffusion, E-test MICs are unaffected by drug properties such as molecular weight, aqueous solubility as well as diffusion characteristics or by varying growth rates of different fungi [8]. In this study, we will adopt the agar based diffusion (ABDD) method and the E-test method to assess the occurrence of resistance in Trichophyton species against Fluconazole, Itraconazole, Terbinafine and Griseofulvin. The criteria for IZDs as to whether a particular isolate is resistant, intermediate or sensitive will be based on a criteria used by R. K. Agarwal et al. (2015) in their article on Antifungal Susceptibility Testing of Dermatophytes by Agar Based Disk Diffusion Method.

2. Materials and Methods

This study was carried out in the Department of Medical Microbiology and Department of Dermatology in the J.S.S Medical College and Hospital, Mysore, between December 2017 and December 2018. All clinically suspected cases of dermatophytosis attending to the Dermatology department became part of the sample provided that they have given oral consent only. Samples were collected after obtaining that informed oral consent from the patients. The study was also approved by the ethical committee of JSS Medical College.

Specimen collection

Suspected lesions were cleaned with 70% ethyl alcohol to remove any dirt and contaminating bacteria. Skin scales and crusts were collected from the erythematous, peripheral, actively growing margins of the lesions by scraping across the inflamed margin of the lesion into the apparently healthy tissue using the blunt edge of a sterile surgical blade onto clean glass slides. Hair specimens were collected by using epilating forceps to pluck along the base of the hair shaft, and scales were scraped from the surface using the blunt edge of a sterile surgical blade. The cutting of hair was avoided as the infection is usually confined to the root, very near the scalp’s surface. Specimens were collected and sealed in sterile dry Petri dishes; they were labeled with the patient’s name, age, sex, date of collection, and site of infection and subsequently brought to the laboratory for mycological examination. The samples were divided into two portions: one for microscopic examination and one for culture.

Microscopic examination and fungal culture

For direct microscopy, the samples collected were screened for the presence of fungal elements using a 10%, 20% and 40% KOH mount is used. Two to three drops of the KOH were kept on a clean, grease-free glass slide. The sample (skin scraping or hair plucking) was placed in the KOH on the slide, and a clean cover slip was placed on the sample and pressed to prevent the formation of air bubbles. The sample was kept in KOH and then observed after 5 - 8 minutes. KOH increases the sensitivity of the preparation and softens keratin. Each slide was
thoroughly examined under low power (10×) and high power (40×) magnification for the presence of hyphae and/or arthroconidia. On the surface of the shaft of infected hairs, the mosaic arrangement of spores was seen (ectothrix infection) or hyphal fragments and arthroconidia was seen internally (endothrix infection). After a direct microscopic examination, irrespective of the demonstration of fungal elements, hair or skin scraping specimens were inoculated in to Sabouraud dextrose agar (SDA), Sabouraud dextrose agar with antibiotics (SDAac) base and the other in a Dermatophyte test Medium agar base both supplemented with chloramphenicol (acts as a broad spectrum antibiotic, which inhibits a wide range of gram-positive and gram-negative bacteria) and cycloheximide (to inhibit saprophytic fungi). To prepare selective media, one vial of Dermatophyte Selective Supplement DS0075 was added to 500 ml of the medium, resulting in concentrations of 0.4 g/l of cycloheximide and 0.05 g/l of chloramphenicol. Cultures were incubated aerobically at room temperature (25˚C) for up to 4 weeks. Positive cultures were examined both macroscopically (color of the surface and reverse, topography, and texture) and microscopically (two types of conidia were formed by dermatophytes: small unicellular microconidia and larger septate macroconidia) for species identification. In the absence of any growth after 4 weeks, the culture was considered.

Anti-fungal susceptibility (ABDD and E-test)

Preparation of inoculum: The isolated fungal colonies were transferred into 5 ml of sterile saline (0.9%), and the suspensions were made by gently probing the surface with the tip of a sterile Pasteur pipette. Heavy particles of the suspension, when present, were allowed to settle for 15 minutes at room temperature and the upper homogenous suspension was used for further testing. The suspensions were mixed with a vortex mixer for 15 seconds and adjusted with sterile normal saline to match the opacity of 0.5 McFarland’s standard.

Agar based disc diffusion method: The antifungal susceptibility testing were performed according to the National Committee for Clinical Laboratory Standards NCCLS (M38-A) guidelines. The isolated dermatophytes were sub-cultured on potato dextrose agar and incubated at 28˚C to enhance sporulation for one week. Following growth, conidia were harvested in sterile saline and conidial suspension was adjusted to 10 × 10⁶ using hemocytometer. Only two Antifungal discs were tested against the dermatophyte isolates. The discs used were Itraconazole (10 mg) and Fluconazole (25 mg). Muller Hinton Agar (MHA) plates were streaked evenly in three directions with a sterile cotton swab dipped into the standardized inoculums suspension. Plates were allowed to dry then antifungal discs were placed onto the medium. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center as illustrated by Figure 1 and Figure 2. The agar plates were then incubated at 37˚C. After 3 to 5 days of incubation, each plate was examined. The zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete
inhibition were measured with an inhibition zone scale.

**E-test**

The E-test method was performed according to the manufacturer’s protocol. The isolated dermatophytes were sub-cultured on potato dextrose agar and incubated at 28°C to enhance sporulation for one week. Four Antifungal agents were tested against the dermatophyte isolates. The antifungal agents were Itraconazole (0.02 - 32 mcg/ml), Terbinafine (0.02 - 32 mcg/ml), Griseofulvin (0.02 - 32 mcg/ml) and Fluconazole (0.016 - 256 mcg/ml). Muller Hinton Agar (MHA) plates were streaked evenly in three directions with a sterile cotton swab dipped into the standardized inoculums suspension. Plates were allowed to dry then antifungal E-strips were placed onto the medium with a pair of forceps. The plates were incubated at 28°C and the results were read at 72 hours for *T. mentagrophytes* and 96 hours for other all species. The E-strips were aseptically removed from the

![Figure 1](image1.png)

**Figure 1.** Sensitive *T. rubrum* to TRB and FLC.

![Figure 2](image2.png)

**Figure 2.** Resistant *T. rubrum* to TRB and FLC.
packages and place on a dry clean surface of MHA plate to, and that the concentration maximum is nearest the rim of the plate. The whole length of the strip is to be in complete contact with the agar surface as illustrated by Figure 3 and Figure 4. The inhibition ellipse will form because the antifungals will diffuse across the porous paper strip. If air pockets were seen underneath the strip, they were removed by pressing gently onto the strip (without moving the strip) with a pair of forceps, moving from the minimum concentration upwards. Small bubbles under the strip would not affect the results. Once applied, the strip was not to be moved because of the instantaneous release of drug into the agar. The IZD were recorded in the results section.

**Determination of MIC endpoints**

In general, MIC was defined as the lowest drug concentration at which the border of the elliptical inhibition zone intercepted the MIC scale on the E-test strip. When a double halo of growth was observed, the MIC was read at the point where growth was completely inhibited. When different intersections were observed on either side of the strip, the highest MIC value was read and recorded in Table 7.

**3. Results**

In the present study, a total of 100 clinically suspected cases of nail, skin and hair dermatophytosis were included in the study. Out of the 100 samples 85 (85%) were skin scrappings, 10 (10%) were hair and 5 (5%) were nail samples as illustrated in Table 1. Furthermore, 56 (56%) samples were collected from male patients and 34 (34%) samples were collected from female patients. The samples in this study were from patients in the age group of 31 - 40 (27%), 51 - 60 (13%), 41 - 50 (13%), 11 - 20 (12%), 1 - 10 (11%), 21 - 30 (10%), 61 - 70 (9%), 71 - 80 (4%)

![Figure 3. Sensitive T. rubrum.](image-url)
and 81 - 90 (1%). All the samples were subjected to KOH microscopy and culture. Out of 100 samples 28 (28%) of them were KOH positive and as well as culture positive, 3 (3%) were KOH negative and culture positive, KOH positive and culture negative were 5 (5%). However, 64 (64%) samples were both KOH and culture negative as illustrated in Table 2. Table 3 illustrates that tinea corporis were the highest at 64% of the identified isolates while tinea unguium and tinea faciei made up 5% each. Table 4 shows the number of respective dermatophytes

Table 1. Sample-wise distribution.

| Sample  | Skin | Hair | Nail |
|---------|------|------|------|
| Number  | 85   | 10   | 5    |
| Percentage % | 85    | 10   | 5    |

Table 2. Direct microscopy and culture.

| Total samples (100) | KOH positive | KOH negative | Total |
|---------------------|--------------|--------------|-------|
| Culture positive    | 28 (28%)     | 3 (3%)       | 31 (31%) |
| Culture negative    | 5 (5%)       | 64 (64%)     | 69 (69%) |

Table 3. Clinical type distribution of samples.

| Sample         | No of samples | Percentage % |
|----------------|---------------|--------------|
| Tinea corporis | 64            | 64           |
| Tinea cruris   | 6             | 6            |
| Tinea pedis    | 10            | 10           |
| Tinea faciei   | 5             | 5            |
| Tinea capitis  | 10            | 10           |
| Tinea unguium  | 5             | 5            |

Figure 4. Resistant T. rubrum.
as identified. **Table 5** was derived from R. K. Agarwal *et al.* (2015) and was used as the criteria for classifying the isolates’ IZD as resistant, intermediate or sensitive while **Table 6** shows MICs criteria as provided by Himedia. The IZD were then carefully recorded in **Table 7, Table 8 and Table 9** respectively.

**Table 4.** Number of organisms isolated.

| Organism          | Number of organisms | Percentage% |
|-------------------|---------------------|-------------|
| *T. rubrum*       | 11                  | 35          |
| *T. mentagrophyte*| 8                   | 25          |
| *T. tonsurans*    | 5                   | 16          |
| *T. verrucosum*   | 2                   | 6           |
| *M. gypseum*      | 3                   | 10          |
| *T. violacium*    | 1                   | 3           |
| *M. audoinii*     | 1                   | 3           |

**Table 5.** Criteria cut off values for IZDs for each of the four drugs*.

| Drugs | Inhibition zone diameters | Sensitive Mean ± 1 SD | Intermediate Sensitive Mean-1 SD to Mean-2 SD | Resistant Mean-2 SD |
|-------|---------------------------|-----------------------|-----------------------------------------------|---------------------|
| FLC   | 22.6±4.2                  | >19                   | 14-19                                         | <14                 |
| ITR   | 27.3±6.2                  | >21                   | 15-21                                         | <15                 |
| TER   | 32.1±6.1                  | >26                   | 20-26                                         | <20                 |
| GRI   | 35.9±4.9                  | >31                   | 26-31                                         | <26                 |

*Criteria provided by Himedia. ABDD: Agar-based disk diffusion; ITR: Itraconazole; FLC: Fluconazole; IZD: Inhibition zone diffusion; S: Sensitive.

**Table 6.** Interpretative criteria for susceptibility categorization for MIC*.

| Interpretative criteria | Drug | <S | S-DD* | >R |
|-------------------------|------|----|------|----|
| FLC                     | 8    | 16 | 2-32 | 64 |
| ITR, TRB, GRI           | 0.125-1 | 02 - 4 | 8   |

*Criteria provided by Himedia. ABDD: Agar based diffusion; FLC: Fluconazole; IZD: Inhibition zone diameter.

**Table 7.** E-test values for the ITR and FLC by average.

| Dermatophyte          | Average IZD (mm) | Average MIC (mcg/ml) |
|-----------------------|------------------|----------------------|
|                       | FLC  | ITR  | FLC  | ITR  |
| *T. rubrum*           | 29.5 | 20.9 | 24   | 0.28 |
| *T. mentagrophyte*    | 36.3 | 29.23| 1    | 0.12 |
| *T. tonsurans*        | 32.6 | 30.4 | 2.2  | 0.142|
| *T. verrucosum*       | 34.5 | 26   | 0.048| 26   |
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| Dermatophyte | ITR | Average IZD (mm) |
|--------------|-----|-----------------|
| *T. rubrum*  | 268 | 24.36363636 S    |
| *T. mentagrophyte* | 233 | 29.125 S        |
| *T. tonsurans* | 152 | 30.4 S         |
| *T. verrucosum* | 52  | 26 S          |
| *M. gypsium* | 81  | 27 S         |
| *M. violacium* | 28  | 28 S         |
| *M. audoinii* | 40  | 40 S         |

Table 8. ABDD values for the ITR and FLC by average.

| Dermatophyte | FLC | Average IZD (mm) |
|--------------|-----|-----------------|
| *T. rubrum*  | 311 | 28.3 S         |
| *ST. Mentagrophyte* | 256 | 32 S         |
| *T. tonsurans* | 163 | 32.6 S        |
| *T. verrucosum* | 69  | 34.5 S        |
| *M. gypsium* | 105 | 35 S         |
| *M. violacium* | 40  | 40 S         |
| *M. audoinii* | 31  | 31 S         |

Table 9. ABDD values for FLC by average.

4. Discussion

Superficial cutaneous fungal infections are commonly encountered fungal diseases prevalent in most parts of the world [8] [16]. The dermatophytes are by far the most significant cutaneous fungi because of their widespread involvement of population at large and their worldwide prevalence [16] [17]. Dermatophytoses form over 50% - 75% of all the mycological infections. The diagnosis of a dermatophytic infection is mostly done clinically, but often confused with other skin infections due to the topical application of steroid ointments and creams, leading to further misdiagnosis and mismanagement [18]. Hence, there arises the need for the correct, efficient, and rapid laboratory diagnosis of dermatophytes [5] [19]. Another important point to consider is that resistance to antifungals has started appearing in dermatophytes [20]. The establishment of a reference antifungal susceptibility testing method may allow the clinician to select the appropriate therapy for the treatment and also for studying mechanisms of
drug resistance of dermatophytic fungi [21] [22].

In the present study a total of 100 specimens (skin scrapings, hair fragments and nail clippings) were collected from clinically suspected cases of dermatophytosis. 69% were from males and 31 (31%) were from female patients visiting the dermatology department. Out of the 100 clinical cases, tinea corporis accounted for 64%, tinea cruris accounted for 6%, tinea pedis 10%, tinea faciei 5%, tinea capitis 10% and tinea unguium accounted for 5% and. In a study done by Bindu et al. in (2016), tinea corporis accounted for 35 (23%) of the cases, tinea pedis in 16 (10%) cases, tinea unguium 5 (10%) cases, tinea capitis in 10 (6%) cases, tinea faciei in 5 (3%) cases and least cases were tinea barbae 2 (1%). A study by Anupama. A et al. (2017) had similar findings in which out of 100 cases male proportion were 73 (73%) and 27 (27%).

The specimens were examined by direct microscopic examinations by potassium hydroxide (KOH) wet mount and by culture methods. Out of a 100 samples, 64% were KOH negative and 36% were KOH positive. Out of 100 samples 31 (31%) were culture positive and 69 (69%) were culture negative, 28 (90.3%) were both culture and KOH positive, 3 (9.6%) were KOH negative and culture positive and 5 (16%) were KOH positive and culture negative. Anupama. A et al. (2017) also reported similar proportions whereby out of 100 clinical samples 58% samples were culture positive and KOH positive, 19% samples were Culture positive and KOH negative, 18% samples were Culture as well as KOH negative. 5% samples were KOH positive and culture negative.

In culture, the isolated dermatophyte isolates were identified by macroscopic morphological characteristics (pigmentation, growth rate and texture etc.) and followed by microscopic examination. The isolated organisms were identified as Trichophyton rubrum 11 (35%), Trichophyton mentagrophyte 8 (25%), Trichophyton tonsurans 5 (16%), Microsporum gypseum 3 (10%), Trichophyton verrucosum 2 (6%), Trichophyton violaceum 1 (3%) and Microsporum audouinii 1 (3%). In a study done by Keyvan Pakshir et al. in (2009), similar findings were reported but in their study Trichophyton mentagrophytes accounted for 13 (32.5%), T. rubrum 8 (20%), T. violaceum 4 (10%), Microsporum gypseum 3 (7.5%), T. tonsurans 2 (5%), T. verrucosum 2 (5%) respectively.

The 31 isolated dermatophytes were tested for antifungal susceptibility testing by Agar based disc diffusion (ABDD) with FLC (25 mcg/ml) and ITR (10 mcg/ml). Out of 31 dermatophytes 25 (80%) isolates were sensitive to both FLC and ITR, 5 (16%) strains were intermediate within the range of (15 - 20 mm) for ITR and FLC (14 - 19 mm) respectively. 1 (4%) of the isolates were resistant to both ITR (14 mm) and FLC (6 mm). The 31 dermatophytes were further tested for antifungal susceptibility testing by E-test with four antifungal agents namely Fluconazole (0.016 - 256 mcg/ml), Itraconazole (0.002 - 32 mcg/ml), Terbinafine (0.002 - 32 mcg/ml), and Griseofulvin (0.002 - 32 mcg/ml). Out of 31 dermatophytes 17 were sensitive to all four antifungal agents within the range of FLC (2 - 6 mcg/ml), ITR (0.125 - 2), TER (0.125 - 2), and GRI (0.125 - 2), 5 isolates were
Resistant in which 2 were resistant to FLC (64; 256 mcg/ml), 2 isolates were resistant to TER (32; 38 mcg/ml), 1 isolate was resistant to both GRI and TER (16; 32 mcg/ml) and 9 isolates were within the intermediate range.

A comparison was made between the two antifungal agents used in both tests (E-test and ABDD). The MIC and IZD values produced by the organism were recorded in Table 4, Table 8 and Table 9. The MIC and the IZD values were found to be inversely proportional to each other [23]. In a study done by Agarwal et al. (2015), similar IZD values of FLU (13 - 30 mm), ITR (20 - 35 mm) were also been reported. In our study the highest MIC values for FLC were (32 - 64 mcg/ml). Sanjivan et al. (2015) also reported similar MIC values of FLC (34 mcg/ml). However, it is difficult to compare results of the E-test and ABDD methods due to variability in critical technical factors in different studies, including inoculums size, type of media, incubation temperature and time of reading, which may explain the different results in antifungal susceptibility testing obtained by various investigators and laboratories [24].

The zones of inhibition were seen in all the strains except 1 strains of T. rubrum as could be seen in Figure 1 and Figure 2. The zone of inhibition varied from 0 > 40 mm for Fluconazole. In E-test, the MIC varied from 256 - 0.125 mcg/ml for Fluconazole, 2 - 4 mcg/ml for Itraconazole, 0 - 48 mcg/ml for Terbinafine and 0 - 3 mcg/ml for Griseofulvin. In a study conducted by Shalini Gupta et al. (2015) similar MIC values in close ranges were reported of four antifungal agents, FLC (32 - 64 mcg/ml, ITR (4 - 6 mcg/ml). Although some in vitro antifungal susceptibility tests are now available including those mentioned in the CLSI document regarding filamentous fungi (CLSI, 2008, 2010, no simple reference method has been standardised for testing the drug susceptibility of dermatophytes) [25] [26].

Experience in determining MICs and careful attention to procedural details are critically important in conducting the E-test method because it is not as easy as it is with inhibition zone scale in disk diffusion method [2] [27]. The MICs and IZDs are inversely proportional to each other i.e. when the MIC for the drug is more; the IZD is smaller and vice versa. Successful treatment of fungal infections depends on the ability of a given antimycotic agent to eradicate the fungus from the tissue [28]. Though some in-vitro antifungal susceptibility tests are now available, no simple reference method has been standardized for testing the drug susceptibility of dermatophytes [28] [29].

5. Conclusions

The present study demonstrated a good correlation between MICs and IZDs of the drugs. It was observed that if the MIC value was low for a particular isolate a larger zone of inhibition was observed [30]. Furthermore, it was also observed that if MIC was higher, a smaller zone of inhibition was observed.

Disc diffusion method is simple, reproducible, cheap and easily adaptable. Furthermore, it has potential for use in selection of appropriate antifungal agents.
once the conditions such as temperature and inoculums size are properly stan-
dardized [31] [32]. The inhibition zones for the disks were easy to measure with
the inhibition zone scale compared to the reading of the E-strips. In addition to
that, more than one antifungal can be tested in the same plate at the same time
to conserve time and media [18] [33]. Antifungal susceptibility testing by disk
diffusion can become an important method in treatment of patients with fungal
infections.

Therefore, based on the results of the present study; it could be concluded that
the method for antifungal susceptibility of dermatophytes is the disc diffusion
method as is the simpler, cheap and reliable method in comparison with the
E-test. However, when it comes to sensitivity the E-test supersedes the disc dif-
fusion method. Previous studies have also shown that the E-test method is the
most sensitive method due to the fact that quantitative MICs can be obtained
directly from the E-strip [34] [35]. However, the E-test method is expensive and
difficult in defining the precise borders of the inhibition zones in dermatophytes
[36]. Despite that, the agar based disk diffusion method was found to be favora-
ble in comparison to the E-test method. Future efforts must put more emphasis
on establishing standard interpretive break-points for dermatophytes for li-
censed as well as newly introduced antifungals and correlating them to the clin-
ical outcomes.

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this pa-
per.

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