An Experimental Investigation On Antifungal Susceptibility to Clinical Specimens

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Abstract

Dermatomycosis is a disease with parasites identified with the skin: glabrous skin, hair and additionally nails. Oral treatment of parasitic contaminations in dermatology has become a favored methodology for the administration of these extremely regular conditions. Despite the fact that there are expanding quantities of antifungals accessible for treatment of dermatophytes, a few cases and backslides have been lethargic to treatment. The assurance of growth in-vitro antifungal powerlessness has been accounted for to be significant for the capacity to kill dermatophytes. It is important to perform antifungal vulnerability testing of dermatophytes. E-test (AB Biodisk, Sweden) is a quick, simple toperform in-vitro antifungal powerlessness test. The point of this examination was to explore the weakness of the various types of dermatophyte strains secluded clinical examples to five antifungal specialists utilizing the E-test strategy.

Keywords: *Dermatophytes, antifungal, treatment.*

1. INTRODUCTION

Dermatophytes are a particular gathering of parasites, which impact keratinous tissue of people and different vertebrates, causing shallow contaminations. The creatures have a place with three genera, Trichophyton, Epidermophyton, and Microsporum. Contaminations brought about by these parasites are among the most predominant cutaneous diseases around the world and the ongoing expansion in the quantity of patients with immunocompromised states, for example, AIDS, diabetes mellitus, malignant growth and organ transplantation has given these contaminations more conspicuousness [1-6]. The treatment of dermatophytosis depends on the utilization of skin and foundational antifungal specialists. As of late, various safe and profoundly viable antifungal specialists have been brought into clinical practice. Albeit an expanding number of antimycotics have opened up for the treatment of dermatophytosis, there are reports recommending refractory to treatment or potentially obstruction of dermatophytes to antimicrobial specialists. To anticipate the capacity of a given antimycotic specialist to kill dermatophytes and help overseeing patients, assurance of the in vitro antifungal defenselessness of dermatophytes would be useful in understanding a fizzled or effective treatment. In any case, not all species have a similar helplessness example and it very well might be important to act in vitro vulnerability testing for choice and checking of antifungal treatment. Albeit a reference strategy isn't yet accessible, different procedures have been utilized to test dermatophytes, including stock large scale and miniature weakening strategies, agar weakening and circle dispersion. Be that as it may, these strategies are tedious and work serious, and are not reasonable for the clinical lab. Accordingly, straightforward elective methodologies are required [1, 3, 4, 6-8]. The E-test is a basic, agar- based, quantitative insignificant inhibitory focus (MIC) strategy. The reagent comprises of a flimsy, adjusted plastic strip with a predefined, dramatic and nonstop inclination of antifungal specialist across 15 two-overlap weakenings. The E-test has been sufficiently used to test microorganisms, yeasts and molds. In any case, there is restricted information accessible on the presentation of the E-test for antifungal weakness of dermatophytes [4, 9-11]. The point of this examination was to explore the helplessness of the various types of dermatophyte strains segregated clinical examples to five antifungal specialists (amphotericin B, fluconazole, itraconazole, caspofungin, and ketoconazole) utilizing the E-test strategy. Materials and Methods Strains and Specimens: Sixty-six strains were disconnected from tainted skin and nails in the Microbiology and Clinical Microbiology Department of School of Medicine, Ataturk University. Secludes were gathered over a one-year time frame in Mycology Laboratory. They included T. rubrum, T. mentagrophytes, M. canis, T. tonsurans, E. floccosum and T. violaceum. All strains were

distinguished by standard strategies, which included ID dependent on the perceptible and infinitesimal qualities of the way of life strains. Extra tests incorporated those for the capacity to create a red shade when the strains were developed on Potato Dextrose Agar (PDA) and for the capacity to deliver urease, just as the hair hole test. Strains were put away - 700 C until the hour of utilization, and preceding testing were sub-refined on PDA at 280 C for 15 days to guarantee ideal development qualities [1, 3, 6]. All methodology in the trial convention were affirmed by The Ethics Committee of Medical Faculty.

2. E-TEST METHOD MEDIUM:

The test was acted in RPMI 1640 medium with L-glutamine, in spite of the fact that without bicarbonate (Gibco, New York, USA), pH 7.0 enhanced with 2% glucose, supported 0.165 M morpholinepropanesulfonic corrosive (MOPS) (Fisher Biotech, New Jersey, USA) and 1.8% agar (Difco, Sparks, USA). The 15-cm width petri plates contained RPMI 1640 at a profundity of 4.0 mm [4]. Antifungal Agents: E-test strips were gotten from AB Biodisk (Solna, Sweden) and put away at - 200 C until tests were performed. The focuses examined went from 0.002 to 32.000 µg/mL-1 for amphotericin B, itraconazole, caspofungin, and ketoconazole and 0.016 to 256.000 µg/mL-1 for fluconazole. Technique: All disconnects were tried against five antifungal specialists utilizing the E-test as indicated by the maker's guidelines. The inoculums suspensions were arranged and changed in accordance with 65-70% conveyance at a frequency of 530 nm comparing to a centralization of 105 - 106 cfu/mL-1 checked by quantitative plate tallies. The RPMI agar surface was vaccinated by dunking a sterile swab into the inoculums suspension and streaking it uniformly in three ways. After overabundance dampness was consumed into the agar and the surface was totally dry, an E-test strip was applied to each plate. The plates were hatched at 280 C and the outcomes were perused at 72-96 hour [4]. Assurance of MIC endpoints: when all is said in done, MIC was characterized as the most reduced medication fixation at which the fringe of the circular restraint zone blocked the MIC scale on the E-test strip. At the point when a twofold radiance of development was noticed, the MIC was perused at where development was totally hindered. At the point when various convergences were seen on one or the other side of the strip, the most elevated MIC esteem was perused [4].

3. RESULTS

The secluded dermatophytes were acquired from the toenails 16 (24.2%), feet 33 (50.0%), inguinal locale 7(10.7%), trunk 5 (7.6%) and hands 5 (7.6%). The appropriation of detached species 66 dermatophytes were T. rubrum 43 (65.1%), T. mentagrophytes 7 (10.7%), M. canis 5 (7.6%), T. tonsurans 5 (7.6%), E. floccosum 4 (6.0%) and T. violaceum 2 (3.0%) (Table 1). All strains tried developed well on RPMI glucose, supplement agar plated. They were perused in the E-test strategy following 96 hours of brooding, with the exception of T. mentagrophytes, which required just 72 hours of hatching. Table 2 sums up the in vitro susceptibilities of 66 clinical detaches of dermatophytes to five antifungal specialists as controlled by E-test. The most dynamic specialist against all dermatophytes species was caspofungin with a MIC range (µg/mL-1) (0.02-3, 0.032-4, 0.032-4, 0.125-0.50, 0.25-0.50, 0.125-0.50) and itraconazole with a MIC range (µg/mL-1) (0.038-1.5, 0.094-1.5, 1-32, 0.016-0.50, 0.25-0.50, 0.125-0.50). The most un-dynamic specialist was fluconazole with a MIC range (µg/mL-1) (0,19-48, 2-256, 2-8, 256, 256, 8-24).Test consequences of the helplessness to amphotericin B and ketoconazole were as per the following; individually, 0,012-8, 0,19-8, 0,50-3, 0,125-6, 32, 0,75 and 0,032-8, 0,064-8, 32, 32, 32, 32. As a rule, the types of dermatophytes indicated comparative examples of vulnerability to every antifungal specialist tried. High MIC values were found for some confines, two dermatophytes strains (1 T. rubrum and 1 T. mentagrophytes) had MICs of caspofungine of 32 µg/mL, 16 strains (11 T. rubrum, 4 E. floccosum and 1 T. mentagrophytes) had MICs of Amphotericin B of 32 µg/mL, 53 strains (36 T. rubrum, 5 T. tonsurans, 4 E. floccosum, 2 M. canis, and 6 T. mentagrophytes) had MICs of fluconazole of 256 µg/mL, 2 strains (2 M. canis) had MICs of itraconazole of 32 µg/mL, and 33 strains (18 T. rubrum, 1 T. tonsurans, 4 E. floccosum, 5 M. canis, 2 T. violaceum, and 3 T. mentagrophytes) had MICs of ketoconazole of 32 µg/mL. Table 2 sums up the MIC ranges, focuses restraining half (MIC 50) and 90% (MIC 90) of the detaches of the five antifungal medications against 66 strains ofdermatophytes.

Table 1. Isolated dermatophyte strains in relation to localization

			Localization				
Dermatophytes	n	%	Toe nail	Foot	Inguinal region	Trunk	Hands
T. rubrum	43	65.1%	12	24	4	3	-
T. mentagrophytes	7	10.7%	1	2	-	2	2
M. canis	5	7.6%	1	3	-	-	1
T. tonsurans	5	7.6%		2	3	-	-
E. floccosum	4	6.0%	2	2	-	-	-
T. violaceum	2	3.0%	-	-	-	-	2
Total	66	100.0%	16 (24.2%)	33 (50.0%)	7 (10.7%)	5 (7.6%)	5 (7.6%)

Table 2. Susceptibility data for dermatophytes species against five antifungal agents using the E-test method

Species (n)	Antifungal agent	MIC range*	MIC_{50}	MIC_{90}
T. rubrum (43)	Amphotericin B	0.012-8	0.50	1.5
	Fluconazole	0.19-48	-	-
	Itraconazole	0.038-1.5	0.50	0.19
	Caspofungine	0.02-3	1	0.064
	Ketoconazole	0.032-8	-	-
T. mentagrophytes (7)	Amphotericin B	0.19-8	0.70	4
	Fluconazole	2-256	-	-
	Itraconazole	0.094-1.5	0.25	1.5
	Caspofungine	0.032-4	0.25	2
	Ketoconazole	0.064-8	2	8
M. canis (5)	Amphotericin B	0.50-3	0.50	1
	Fluconazole	2-8	-	-
	Itraconazole	1-32	-	-
	Caspofungine	0.125-0.50	0.50	0.125
	Ketoconazole	32	-	-
T. tonsurans (5)	Amphotericin B	0.125-6	0.50	0.50
	Fluconazole	256	-	-
	Itraconazole	0.016-0.50	0.125	0.125
	Caspofungine	0.032-2	0.032	0.032
	Ketoconazole	32	-	-
E. floccosum (4)	Amphotericin B	32	-	-
	Fluconazole	256	-	-
	Itraconazole	0.25-0.50	0.25	0.50
	Caspofungine	0.25-0.50	0.25	-
	Ketoconazole	32	-	-
T. violaceum (2)	Amphotericin B	0.75	-	-
	Fluconazole	8-24	-	-
	Itraconazole	0.125-0.50	-	-
	Caspofungine	0.125-0.50	-	-
	Ketoconazole	32	-	-

MIC: Minimal inhibitory concentration
*MIC ($\mu g/mL^{-1}$)

4. DISCUSSION

Diseases brought about by dermatophytes happen worldwide and can be extremely serious and hard to treat in patients whose immunological reaction is impeded. These contaminations spoke to a significant general medical issue up 'til now uncertain [4, 7]. Dermatophytes are answerable for most of parasitic contaminations including the skin, hair and nails. They include a phylogenetically firmly related gathering of genera with various species. They assault the keratinized tissues and cause a wide range of clinical appearances that shift from mellow to serious [6]. The appropriation of the dermatophytes and their etiological specialists has inconsistent frequencies, with varieties of their predominance as indicated by the nations and even the areas of a similar nation. In this examination, T. rubrum was the most oftentimes disengaged creature 43 (65.1%), trailed by T. mentagrophytes 7 (10.7%), M. canis 5 (7.6%), T. tonsurans 5 (7.6%), E. floccosum 4 (6.0%) and T. violaceum 2 (3.0%). These outcomes are in concurrence with numerous other nearby [3, 12-19] and worldwide investigations [1, 4, 7, 9, 20-25]. Most shallow diseases brought about by dermatophytes can be quickly annihilated with effective and foundational antifungals. Oral antifungal treatment with more up to date specialists, for example, terbinafine, itraconazole and fluconazole, is the treatment of decision for dermatophytosis that doesn't react to skin treatments. The action range to these medications is variable, prompting treatment disappointment in 25-40% of treated patients, conceivably because of helpless patient consistence, absence of medication infiltration into nail, prescription bioavailability or medication connections and obstruction [26]. In vitro examination of the antifungal movement of hostile to parasitic specialists empowers correlation between various antimycotics, which thus may explain the explanations behind absence of clinical reaction and help clinicians in picking a successful treatment for their patients. Nonetheless, it is significant that the strategies utilized for in vitro testing be normalized to encourage the foundation of value control boundaries and interpretive break focuses [27]. As of now, no reference strategy has been set up to test drug susceptibilities of dermatophytes. The advancement of straightforward and reproducible methods is needed for clinical testing of these significant microorganisms. The E-test is another and promising technique with wide applications in clinical research facility practice, and is upheld by the aftereffects of broad testing of microbes and yeasts. Notwithstanding, there are a couple of reports portraying the utilization of this strategy for dermatophytes [4, 9, 20-22].

In this examination, we explored MIC estimations of five antifungal specialists (amphotericin B, fluconazole, itraconazole, caspofungin, and ketoconazole) to the various types of dermatophyte strains confined clinical examples utilizing the E-test strategy. In our examination, the most dynamic specialist against all dermatophytes species was caspofungin with a MIC range (µg/mL-1) (0.02-3, 0.032-4, 0.125-0.50, 0.032-2, 0.25-0.50, 0.125-0.50) and itraconazole with a MIC range (µg/mL-1) (0.038-1.5, 0.094-1.5, 1-32, 0.016-0.50, 0.25-0.50, 0.125-0.50). The most un-dynamic specialist was fluconazole with a MIC range (µg/mL-1) (0.19-48, 2-256, 2-8, 256, 256, 8-24). Test aftereffects of the vulnerability to amphotericin B and ketoconazole were as per the following; separately, 0.012-8, 0.19-8, 0.50-3, 0.125-6, 32, 0.75 and 0,032-8, 0,064-8, 32, 32, 32, 32. As for itraconazole, the entirety of T. rubrum detaches were restrained in fixations going from 0.038 to 1.5 µg/mL-1. Different species, aside from M. canis indicated comparative affectability ranges. Two M. canis strains had MICs of itraconazole of 32 µg/mL. Notwithstanding, for fluconazole, we saw that high MIC values. 53 strains (36 T. rubrum, 5 T. tonsurans, 4 E. floccosum, 2 M. canis, and 6 T. mentagrophytes) had MICs of fluconazole of 256 µg/mL. By and large, our information are in concurrence with investigations of Don Santos et al. [22], Fernandez-Torres et al. [4], Silva-Barros et al. [9], Kang et al. [21] and Abdel-Aal et al. [20]. Caspofungin the other most dynamic specialists for all dermatophytes species in our investigation with a MIC range (0.02-3 for T. rubrum, 0.032-4 for T. mentagrophytes, 0.032-4 for M. canis, 0.032-2 for T. tonsurans, 0.25-0.50 for E. floccosum, 0.125-0.50 for T. violaceum). In our investigation, 33 (half) disengages of tried dermatophytes by E-test (18 T. rubrum, 1 T. tonsurans, 4 E. floccosum, 5 M. canis, 2 T. violaceum, and 3 T. mentagrophytes) were safe with a MIC range 32 µg/mL of ketoconazole. These outcomes were gotten different analysts [4, 9, 20-22]. Amphotericin B, the other medication with a MIC range (0.012-8, 0.19-8, 0.50-3, 0.125-6, 32, 0.75) in the current investigation. 16 strains (11 T. rubrum, 4 E. floccosum and 1 T. mentagrophytes) had MICs of amphotericin B of 32 µg/mL. Kang et al.[21] saw that amphotericin B was 0.094~0.5 µg/mL on T. rubrum, 0.032~1.0 µg/mL on T. mentagrophytes, 0.19 µg/mL on M. canis, and 0.032 µg/mL on M. gypseum. Antifungal defenselessness testing is a powerful field of clinical mycology. Improvement and normalization of antifungal helplessness test have demonstrated momentous advancement in the field of clinical mycology [6], in spite of the fact that, examines utilizing the E-test technique for dermatophytes susceptibilities isn't yet adequate. In a set number of studies, indicated that E-test is by all accounts an elective technique to MIC-assurance of antifungal medications for dermatophytes, since it is a lesslaborious strategy and results could be acquired quicker.

5. CONCLUSION

In conclusion, this investigation indicated that the E-test spoke to a straightforward and adequate strategy for antifungal helplessness testing of dermatophytes. Concerning execution, the E-test was not work requesting, was anything but difficult to decipher, and with the capability of being utilized as an elective measure for azole antifungal weakness testing of dermatophytes.

6. REFERENCES

1. Ebrahim HM, Asaad AM, Amer A. Antifungal susceptibility patterns of dermatophytes clinical isolates from dermatophytosis patients before and after therapy. Egptian J of Med Microbiol 2010; 19: 41-46.
2. Fernandez- Torres B, Carrillo AJ, Martin E, et al. In vitro activities of 10 antifungal drugs against 508 dermatophytes strains. Antimicrob Agents and Chemother 2001; 45: 2524-8. [CrossRef]
3. Karaca N, Koç AN. In vitro susceptibility testing of dermatophytes: comparison of disk diffusion and reference broth dilution method. Diagn Microbiol Infect Dis 2004; 48: 259-64. [CrossRef]
4. Fernandez- Torres B, Carrillo-Munoz A, Ortoneda M, et al. Interlaboratory evaluation of the E-test for antifungal susceptibility testing of dermatophytes. Med Mycol 2003; 41: 125-130. [CrossRef]
5. Fernandez-Torres B, Carrillo-Munoz A, Inza I, et al. Effect of culture medium on the disk diffusion method for determining antifungal susceptibilities of dermatophytes. Antimicrob Agents and Chemother 2006; 50: 2222-4. [CrossRef]
6. Pakshir K, Bahaedinie L, Rezaei Z, et al. In vitro activity of six antifungal against clinically important dermatophytes. Jundishapur Journal of Microbiology 2009; 2: 158-163.
7. Araujo CR, Miranda KC, Fernandes OFL, et al. In-vitro susceptibility testing of dermatophytes isolated in Goiana, Brazil, against five antifungal agents by broth microdilution method. Rev Inst Med Trop Sao Paulo 2009; 51: 9-12. [CrossRef]
8. Siqueira ER, Ferreira JC, Pedrosa RS, et al. Dermatophyte susceptibilities to antifungal azole agents tested in vitro by broth macro and microdilution methods. Rev Inst Med Trop Sao Paulo 2008; 50: 1-5. [CrossRef]
9. da Silva Barros ME, de Assis Santos D, Soares Hamdan J. Antifungal susceptibility testing of trichophyton rubrum by E-test. Arch Dermatol Res 2007; 299: 107-9. [CrossRef]
10. Pfaller MA, Messer SA, Mills K, et al. In-vitro Susceptibility Testing of Filamentous Fungi: comparison of E-test and Reference Microdilution Methods for Determining Itraconazole MICS. J Clin Microbiol 2000; 38: 3359-61.
11. Favel A, Michel-Nguyen A, Chastin C, et al. In-vitro susceptibility patterns of candida lusitaniae and evaluation of the E-test method. J Antimicrob Chemother 1997; 39: 591-6. [CrossRef]
12. Sarifakioglu E, Seçkin D, Demirbilek M, et al. In vitro antifungal susceptibility patterns of dermatophyte strains causing tinea unguium. Clin Exp Dermatol 2007; 32: 675-9. [CrossRef]
13. Çetinkaya Z, Kiraz N, Karaca S, et al. Antifungal susceptibilities of dermatophytic agents isolated from clinical specimens. Eur J Dermatol 2005; 15: 258-61.
14. Bilgili ME, Sabuncu İ, Saraçoğlu ZN, et al. Dermatophyte types isolated from patients presented with dermatophytosis in our clinic. Turkiye Klinikleri J Dermatol 2001; 11: 185-90.
15. Dilek N, Yücel AY, Dilek AR, et al. Dermatophytosis agents in patients who attending to dermatology clinic of Fırat University Hospital. Turkish J of Dermatol 2009; 3: 27-31.
16. Gürcan Ş, Tikveşli M, Eskiocak M, et al. Investigation of the agents and risk factors of dermatophytosis: a hospital-based study. Mikrobiyol Bul 2008; 42: 95-102.
17. İnci M, Özer B, Duran N, et al. Onikomikoz ön tanısıyla gönderilen örneklerden izole edilen dermatofitlerin değerlendirilmesi. Türk Mikrobiyol Cem Derg 2011; 41: 61-64.
18. Özekinci T, Özbek E, Gedik M, et al. Dicle Üniversitesi Tıp Fakültesi mikrobiyoloji laboratuvarına başvuran hastalarda dermatofitoz etkenleri. Dicle Tıp Dergisi 2006; 33: 19-22.