Current trends of antifungal susceptibility pattern of dermatomycosis in a tertiary care hospital by Etest and VITEK-2 methodologies

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Abstract
Dermatomycosis affect the superficial layers of the skin, nails and hair and are often caused by dermatophytic molds, candida & non dermatophytic molds. However, over the last decade, an increasing number of non– dermatophyte filamentous fungi & non albicans Candida have been recognized as agents of skin and nail infections in humans, producing lesions clinically similar to those caused by dermatophytes. Although tissue invasion by them is a rare possibility, they are important as a public health problem particularly in the immunocompromised. There are limited studies on the efficacy of antifungal agents against dermatophytes. This study was conducted to test the efficacy of 3 systemic antifungal agents viz. Voriconazole, Itraconazole & Fluconazole using the E-test method and 2 systemic antifungals viz. Itraconazole and Voriconazole by VITEK-2. Three different species of dermatophytes which were isolated from the clinically suspected cases were Trichophyton mentagrophytes, T.rubrum and M.gypseum. According to the obtained results, Itraconazole and Voriconazole showed the lowest MIC range while Fluconazole had the highest MIC range for most fungi tested. Determining the resistance pattern is especially necessary to assist clinicians in treating superficial fungal infections more effectively. This will minimize side effects and prevent development of antifungal resistance & treatment failures.

Keywords: Dermatomycosis, Antifungal resistance, Etest, VITEK-2, Voriconazole, Itraconazole, Fluconazole.

Introduction

Dermatomycosis or superficial fungal infections are among the most common diseases seen in daily dermatological practice. High environmental temperature in the tropical and the subtropical regions, poor personal hygiene, poor nutrition tight clothing, overcrowding, debilitating systemic diseases like diabetes, leukemia and other endocrine disorders, drug resistance, immunocompromised states like HIV infection etc. have contributed to make it rampant.

Few of these fungi have acquired the skill to digest keratin, playing role as a keratinolytic agent in biodegradation. The moulds causing best decay of keratin are *Microsporum*, *Trichophyton* and *Epidermophyton*. Superficial fungal infections can also be caused by by *Pityriasis versicolor*, candida & non dermatomycotic molds.[1,2] The presence of these genera presents health issues in developing countries where they cause the mortal mycotic contagion. Diseases caused by fungi, or mycoses, can be clinically classified as superficial, deep, or systemic. Dermatophytes are the most important microorganisms, which cause superficial mycosis, and the lesions are characterized by circular configuration, desquamation and erythema of the edges. The dermatophytes have the capacity to invade keratinized tissue (skin, hair and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm.[3]

Fungal infections are a major cause of morbidity and mortality despite the latest developments of diagnostic tools and therapeutic options. Early initiation of the correct antifungal therapy has been demonstrated to have a direct impact on the patient's outcome. Azole antifungals are quite frequently used in the clinical setting to treat fungal infections. These include Itraconazole, Fluconazole, Voriconazole, Posaconazole, Isavuconazole. The availability of new antifungals in recent years has provided clinicians with more options, increasing the use of these compounds not just for treatment when the infection has been diagnosed, but also as prophylactic, empirical or preemptive treatment. The increased use of antifungals has induced a higher selective pressure on fungal strains and resistance has emerged in two main ways: several species have developed secondary resistance and susceptible species have been replaced by resistant ones, changing the epidemiology of fungal infections.[4]

Antifungal susceptibility testing methods are available to detect antifungal resistance and to determine the best treatment for a specific fungus. Microdilution methods are the gold standard or reference techniques. Regardless of their advantages, the standardized broth microdilution methods of antifungal susceptibility testing are time-consuming and cumbersome for clinical laboratories. Some commercially available methods like the Etest, do not require complex handling and are cost-effective alternative methods to test antifungal agents.[5]

Another commercially available system, VITEK-2 yeast susceptibility test (bioMérieux, Inc.) is an automated method of yeast species identification and antifungal susceptibility testing through the analysis of yeast growth. The system is a miniaturized version of the broth dilution method. The system integrates a

software program which validates and interprets susceptibility test results according to CLSI clinical breakpoints based on the drug MIC values.[5]

The increase of resistant strains associated with treatment failure highlights the need of antifungal resistance surveillance, which should ideally be made in reference laboratories using reference procedures. Hence, a study was undertaken in the Department of Dermatology in pivotal association with the Department of Microbiology of a tertiary care centre to study the current trends and susceptibility pattern of dermatomycosis.

Materials and Methods

A prospective study was conducted in the Department of Dermatology of a tertiary care center over a period of two years. A total of 240 patients were included. An approval from the institutional ethics committee was taken. Data was collected in a predesigned format. For patients with sufficient scales, specimen collection, processing, microscopy and culture were done and antifungal susceptibility testing was carried out as per E-test.

E-Test: Isolates of dermatophytes were tested along with Trichophyton rubrum ATCC 28188 and Trichophyton mentagrophytes ATCC 9533 as control. Dermatophytes 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 $1x10^{6}$ /ml using a haemocytometer. Plates of Mueller Hinton Agar (MHA) were inoculated using a swab dipped in the inoculum suspension. The inoculated plates were then dried before applying the E-strips.

Commercially available E-strips (HIMEDIA) were used to detect the susceptibility of various dermatophytes isolated, to Fluconazole, Itraconazole and Voriconazole.

Sterile disks were also impregnated with 10 l of 1:100 dilution of DMSO to serve as control. The E-strips for the aforementioned 3 drugs were applied to each inoculated & dried plate & then incubated at 28°C for up to 16 hours or longer for filamentous fungi depending on the fungus' genus for the E-strips. When growth took place, the size of zones of inhibition were measured for each antifungal agent as was done by Keyvan P et al in their study.[6]

VITEK-2: Automated Vitek 2 was utilized for confirmation of the Candida species and for testing their susceptibility to Voriconazole and Fluconazole.

Data Analysis: MIC range was obtained and compared for all the isolates tested with lower range implying a higher susceptibility.

Results and Discussion

A total of 240 clinically suspected cases of superficial fungal infections who visited the dermatology outpatient department were selected for microbiological diagnosis.

Of these 240 patients, 180(75%) belonged to rural areas and 60(25%) hailed from urban areas. On analyzing the literacy level of the patients, it was found that the majority of them i.e. 150 (62.5%) were illiterate.

The gender distribution revealed a higher occurrence in male patients who were 142 (59.1%) compared to female patients who were 98(40.9%), with a male is to female ratio of 1.5:1. Our finding of male predominance is quite similar to the findings of the studies conducted by Mahajan S et al in the year 2017, Kumar S et al in the year 2014 and Kumar Y et al in the year 2015 who showed a male: female ratio of 3:1, 2:1 and 2.17:1, respectively.[7-9] Whereas, the study conducted by Bhatia et al in Himachal Pradesh showed a much higher M:F ratio of 5.7:1.[10] Male predominance could be due to the fact that males are more involved in outdoor physical activities, that lead to excessive sweating providing a favorable environment for fungal infections. Both Prabhu et al and Ali AM et al, however, observed a female predominance in their studies, particularly in fungal infections of feet (T. pedis), hands (T. manuum) and nails (onychomycosis, their commonest clinical variant) possibly due to a higher indulgence of females in kitchen and household work.[11,12]

Most of the infected patients were aged between 21 and 40 years being 123 (51.25%). The Age wise distribution was observed as shown in Table 1.

Table 1: Age-wise distribution of patients

Age Group (in years)	No. of patients (Total 240)
0-20	36 (15%)
21-40	123 (51.25%)
41-60	51(21.25%)
61-80	22(9.17%)
81-100	8(3.33%)

Similar was the finding of the study conducted by Jha et al in 920 patients in Mysore, where the most common age group afflicted was 26 to 30 years.[13] While Prabhu et al in their study in 96 patients, showed 30 to 45 years to be the commonest age group affected.[11] This age group comprises of relatively younger individuals who are most actively involved in income generation and thus in outdoor activities leading to their higher exposure to environments that are conducive to the development of superficial fungal infections.

The commonest clinical presentation was a combination of Tinea corporis and cruris (90, 37.5%). Other presentations included Tinea corporis (42, 17.5%), Tinea cruris (33, 13.75%), Onychomycosis (24,10%), Candidal intertrigo (14, 5.83%), Candidal vulvovaginitis (9,3.75%), Tinea pedis (12,5%), Tinea capitis (9, 3.75%), Tinea faciei (7, 2.92%). (Table 2)

Table 2: Distribution of patients according to clinical presentation

Clinical presentation	No. of patients (Total 240)
Tinea corporis and cruris	90 (37.5%)
Tinea corporis	42 (17.5%)
Tinea cruris	33 (13.75%)
Onychomycosis	24 (10%)
Candidal intertrigo	14 (5.83%)
Candidal vulvovaginitis	9 (3.75%)
Tinea pedis	12 (5%)
Tinea capitis	9 (3.75%)
Tinea faciei	7 (2.92%)

There is minimal data with simultaneous Tinea corporis & cruris infection as the most common presentation. This occurrence is probably due to poor hygiene and the practice of touching different parts of the body after scratching the site of primary infection with the same hand (autoinoculation). Findings of the study by Kumar et al in the year 2015 and Madhulika et al in 2014 revealed T. corporis (43%, 58.48% respectively) as the most common clinical presentation. Whereas simultaneous manifestation of T.corporis and T. cruris (3.62%) was found to be much less common by the latter.[9,14] Kainthola et al and Gupta et al showed an almost similar prevalence of T. corporis (28.38% and 25% respectively) as against T. capitis (43.24%) which was the most common presentation in the study conducted by the former, and T. unguium (52%) which was the commonest presentation in the study by the latter.[15, 16]

The distribution of samples obtained from skin, hair and nails was 207 (86.25%), 9 (3.75%) and 24 (10%) respectively.

The total number of positive cultures was 59. Final strain identification revealed 41(69.49%) dermatophytes, 11(18.64%) non dermatophytic molds (NDM) and 7 (11.87%) yeasts (candida). Candida was the commonest species identified among non-dermatophytes (7 Candida sp. out of 18 non-dermatophytes), similar to the findings of the study conducted by Lakshmanan et al.[17] Similar observations were also made in the study conducted by Mohanty et al in which out of 228 specimens subjected for culture, dermatophytes were isolated in 85 cases and Candida species in 9 cases.[18]

The various species were identified on the basis of direct microscopy and culture, with 30 (12.5%) samples positive on both microscopic examination and culture whereas 29 (12.08%) samples positive only on culture. One hundred six (44.17%) samples were positive only on microscopy and 75 (31.25%) samples were found to be negative on both. These 181 culture negative samples could not be processed for antifungal susceptibility testing. The total KOH positivity was found out as 56.67% while the total culture positivity rate was only 24.58%. The 7 (11.87%) strains identified as yeasts (Candida) were confirmed by the Automated VITEK-2. Candida species was conspicuously the most common of the non dermatophytic fungi being 7out of 18 (38.89%).

Similar to our study, the finding of higher positivity of microscopy over culture has been reported by Surekha et al in the year 2015, (potassium hydroxide mount was positive in 77.7% and culture was positive in 30.8%) and Malik A et al (the KOH positivity rate was 61.2% and culture positivity rate was 58.8%).[19, 20]

Thus, the gold standard for the diagnosis of a superficial fungal infection must be a thorough clinical examination followed by a positive KOH smear and a positive fungal culture in 3 weeks. Therefore KOH smear and fungal culture may be considered as complementary laboratory examinations. KOH smear may be used as a good screening test for determining presence of disease, both before and at the end of therapy. A fungal culture could probably serve as a more specific confirmatory test. The empiric treatment with antifungals in the absence of either a positive KOH mount or fungal culture should be discouraged as it involves unnecessary expenditure and chances of missing an alternate diagnosis.

Among the dermatophytes, *Trichophyton* genus represented 97.6% of the isolates, with *T. mentagrophytes* being the commonest that is 25 (60.98 %), followed by *T. rubrum* 15 (36.58%) and *Microsporum gypseum* 1 (2.44%). The non-dermatophytic filamentous fungi constituted 18.64% of the isolates. Among these, the commonest was Aspergillus species (6, 54.55%), followed by Penicillium species (2, 18.18%), Alternaria species (2, 18.18%) and Fusarium species (1,9.09%). Similar to our study, were the findings of the study conducted by Bhatia VK et al in the year 2014, in which *Trichophyton* species were implicated in 98.6% (73/74) cases while *Microsporum* species was detected only in 1.35% cases. Also, none of the *Epidermophyton* species was recovered by them. Further, *Trichophyton mentagrophyte* was also the predominant organism (64.9%) followed by *Trichophyton rubrum* (35.1%).[10] Trichophyton mentagrophyte was also the most common isolate in the study conducted by Sahai et al in the year 2011.[21]

However, many studies have reported *Trichophyton rubrum* as the commonest isolate.[19,22, 23]

In the present study, 7 (11.87%) Candida species were isolated. In the study conducted by Patel et al, non dermatophytic fungus like Candida species were isolated in 8 (10.67%) cases which is quite similar to our findings.[23]

The distribution of species varies from one site to another as shown in Table 3.

Table 3: Most common species in various clinical presentations

Clinical presentation	Most common species
Tinea corporis & cruris	T.mentagrophytes (60%)
Tinea corporis	T.rubrum (54.5%)
Tinea cruris	T.rubrum (66.7%)
Onychomycosis	T.mentagrophytes (33.3%), T.rubrum (33.3%)
Candidal intertrigo	Candida sp (100%)
Candidal vulvovaginitis	Candida sp (100%)
Tinea pedis	T.mentagrophytes (50%)
Tinea capitis	T.rubrum (60%)
Tinea faciei	T.rubrum (60%)

In our study, the variation in species distribution with respect to clinical presentation was found to be statistically significant (p = 0.001). The most common species isolated in Tinea corporis, T.cruris, T.capitis and T.faciei was Trichophyton rubrum. Aly R also found *Trichophyton rubrum* as the commonest cause of Tinea cruris, Tinea corporis and Onychomycosis. The study also stated T.rubrum to be most commonly implicated in causing Tinea pedis which is in contrast to our study (most common cause being T.mentagrophytes).[24] Tah HH also observed Trichophyton rubrum to be the most commonly isolated species from most Tinea infections except in case of Tinea pedis as was observed in our study.[25]

In our study, 41 strains of dermatophytes isolated were tested for their antifungal sensitivity to Voriconazole, Fluconazole and Itraconazole using the E-test method. MIC ranges are as shown in Table 4.

In our study, the MIC range of Voriconazole was from 0.008 µg/ml to 0.064 µg/ml. Results of E-tests for Voriconazole (0.008–0.064 µg/ml) have not been compared because of paucity of relevant data. Although currently Voriconazole is not among commonly prescribed drugs for the management of dermatomycosis, it can be a useful alternative for recalcitrant infections.

All the strains of dermatophytes in our study showed uniform resistance to Fluconazole (i.e. MIC ≥32 µg/ml) when tested by the E strips.

Our findings of poor susceptibility of dermatophytes to Fluconazole by E-test method (Table 4) is compatible with the studies conducted by Favre et al, Santos et al, Barros et al and Sarifakioglu et al.[26-29] Korting et al suggested that high values of MIC for Fluconazole may be due to technical problems, such as interference with some ingredients of the culture media or insolubility at high concentrations.[30] The easy availability of Fluconazole at pharmacies, self medication by patients due to it's over the counter (OTC) preparations available and a rampant practice of its irrational prescription by quacks could be some other reasons for development of resistance to Fluconazole.

In our study, the MIC range of Itraconazole was from 0.016 µg/ml to 0.064 µg/ml. This MIC range was found to overlap with the MIC range of Itraconazole observed by Aktas AE et al (0.038–1.5 µg/ml) in their study. [31] Similar were the results found by Fernandez-Torres et al in their study on the interlaboratory evaluation of E-test for dermatophytes.[32]

As can be interpreted from the table 4, E-test results revealed uniform sensitivity of the three dermatophyte species tested to Voriconazole (MIC range = 0.008-0.064µg/ml) and Itraconazole (MIC range = 0.016-0.064µg/ml) while uniform resistance to Fluconazole (MIC ≥ 32 µg/ml) was noticed in all the three dermatophyte species.

Table 4: E-test result for Voriconazole, Itraconazole and Fluconazole for 3 species of dermatophytes

Drug name	Strain	MIC Range (µg/ml)
Voriconazole	T.mentagrophytes (n = 25)	0.032 – 0.064
	T.rubrum (n = 15)	0.008 – 0.016
	M.gypseum (n = 1)	0.023*
Itraconazole	T.mentagrophytes (n = 25)	0.047 – 0.064
	T.rubrum (n = 15)	0.016 – 0.064
	M.gypseum (n = 1)	0.032*
Fluconazole	T.mentagrophytes (n = 25)	≥32
	T.rubrum (n = 15)	≥32
	M.gypseum (n = 1)	≥32

*Range not applicable

The various species of Candida that were isolated were tested for their antifungal sensitivity to Fluconazole (Table 5) and Voriconazole (Table 6) using the VITEK-2 method.

Table 5: Various species of Candida isolated and their respective MICs (for Fluconazole)

Candida Species	MIC (µg/ml)
C.albicans	≤ 1
C.tropicalis	≤ 1
C.parapsilosis	≤ 1
C.albicans	≤ 1
C.parapsilosis	≤ 1

Table 6: Various species of Candida isolated and their respective MICs (for Voriconazole)

Candida Species	MIC (µg/ml)
C.albicans	≤0.12
C.tropicalis	≤0.12
C.parapsilosis	≤0.12
C.albicans	≤0.12
C.parapsilos	≤0.12
C.tropicalis	≤0.12
C.albicans	≤0.12

The 7 (11.87%) strains identified as Candida were confirmed by the automated VITEK-2 and were found to be uniformly sensitive to Fluconazole (MIC≤ 1µg/ml) and to Voriconazole (MIC≤0.12µg/ml). The high sensitivity of Candida species to Voriconazole (MIC≤0.12 µg/ml) as found by VITEK-2 in our study is in agreement with the observations of the study conducted by Bueno et al who found the lowest MIC values for Voriconazole against Candida species when compared with other commonly used systemic antifungal agents. [33]

In the study by Hazen KC et al, the susceptibility of *C. albicans and C. glabrata* isolates to Fluconazole was 99% and 81.7% respectively whereas Voriconazole demonstrated 10- to 100-fold greater in vitro activity than Fluconazole against most yeast (Candida) species. This is similar to our finding of a 10 fold higher activity of Voriconazole as compared to Fluconazole against various Candida species.[34] In the study by Pfaller MA et al, overall 90.1% of all Candida isolates tested were susceptible to Fluconazole. Of all the isolates of Candida species tested against Voriconazole, 94.8% were sensitive.[35]

In our study, the mean MICs of antifungal drugs did not show statistically significant differences between various species ($p > 0.05$). Hence the treatment of dermatophytic infections need not differ with respect to the clinical variant but should ideally be guided by a culture sensitivity report.

In this study, Voriconazole was found to have the lowest geometric mean while Fluconazole had the highest geometric mean value and MIC range. Therefore, it can be interpreted that Voriconazole being the most sensitive antifungal drug for dermatophytes is a more suitable treatment option but it must be reserved for resistant and difficult to treat cases so as to prevent rapid development of resistance. Itraconazole is a much more affordable antifungal drug that closely follows Voriconazole in its effectiveness against dermatophytes, hence, it must be a preferred treatment option for better outcome in patients suffering from dermatomycosis. Fluconazole being the least sensitive antifungal drug against dermatophytes must be used cautiously due to its poor effect. For Candida species both Voriconazole and Fluconazole were found to be uniformly effective as found by VITEK-2. There is a need for establishing a standard method for antibiogram of dermatophytes to facilitate the selection of drug similar to what is routinely performed for yeasts (candida) and bacteria.

Conclusion

A study was carried out on 240 patients with superficial fungal infections. KOH mount and fungal culture and sensitivity testing was performed for Fluconazole, Voriconazole and Itraconazole. Combined tinea cruris & corporis type was the most common type. Microscopic positivity was found to be higher than culture positivity. Voriconazole and Itraconazole both were found to be highly effective antidermatophytic agents. Fluconazole had a high MIC value against dermatophytes, therefore was found to be a less effective antidermatophytic agent. For Candida species, both Voriconazole and Fluconazole were highly effective drugs as deduced by the results of automated VITEK-2.

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