Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

Antifungal Susceptibility Profile of Dermatophytes in a Tertiary Care Hospital

Satyasai Badveti[1], Ananthraj Tejashree[2*], Veeranna.Sastry[3], Manthravadi Krishna Karthik[4]

1. Tutor & Research Scholar, Dept of Microbiology, JSSMC, JSSAHER, Mysore.
2. Professor& Head, Dept of Microbiology, JSSMC, JSSAHER, Mysore.
3. Professor& Head, Dept of Dermatology, JSSMC, JSSAHER, Mysore.
4. Tutor & Research Scholar, Dept of Microbiology, JSSMC, JSSAHER, Mysore.

(*Tejashree. Ananthraj,Email: dr_tejashree@yahoo.co.in)

ABSTRACT

The main aim of the current research was to detect the species level of dermatophytes. Primary Isolation and speciation of dermatophytes will help us to know the source of infection. Antifungal susceptibility testing of dermatophytes will play a crucial role in understanding a sensitivity patterns and failed or successful in treatment. In the present study 180 clinical suspicions collected from outpatient department of dermatology. The collected clinical suspicions were processed direct microscopy and inoculated onto dermatophyte test medium, Sabouraud dextrose agar containing cycloheximide, potato dextrose agar for isolation. The identified fungal isolates were further tested with four antifungal agents by using two antifungal susceptibility testing methods. In this study out of 180 clinical cases tinea corporis 108(60%) was the most predominant condition followed by tinea unguium 54 (30%). Among 180 samples 96 (53.3%) yielded the growth of dermatophytes in which 45 (46.8%) were *Trichophytonrubrum* followed by 29 (30.2%)*Trichophyton mentagrophytes*. Itraconazole showed the highest rate of sensitivity followed by griseofulvin in both methods.

Keywords:- Dermatophytosis, Dermatophyte test medium, E-Test, Disk diffusion method

Introduction

Dermatophytes, one of the most important members among cutaneous fungi and is known to cause dermatophytosis and is also considered as one of the major public health problems in the world. These fungi cause infections of superficial layers of skin, hair & nails. 3 major genera of dermatophytes include Trichophyton, Epidermophyton and Microsporum. Two major properties of these fungi are keratinophilic and keratinolysis[1]. These fungi have a special ability to break down the keratin and utilize it as the source of energy. According to World Health Organization about 20% of world population is affected by dermatophytes. These infections are more common in India and is more commonly seen among individuals who are actively involved in athletics and sports[2].

Genus *Trichophyton* is the commonest etiological agent isolated among dermatophytosis and especially the species of *Trichophytonrubrum, Trichophytonmentagrophytes* and *Trichophytontonsurans* are identified as the most frequent causative agents of Tineacorporis, Tineacapitis, Tinea ungums, Tineacruris and Tinea.facei[3]. Many studies revealed that diagnosis

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

of dermatophytosis can be done based on the typical clinical presentation, but laboratory identification is essential for the cases with atypical clinical manifestations. Hence, laboratory confirmation plays a crucial role in the management of dermatophytosis in terms of isolation and identification[4].

Drug resistance in dermatophytes in recent times has become a key role in public health problem which damages the quality of life. The inappropriate usage of antifungal agents stays the major cause of antifungal resistance. Early detection of drug resistance and the treatment is essential to reduce the transmission of the infection[5]. Antifungal susceptibility testing and its sensitivity and resistant patterns will help us to know either failed or successful treatment and also helps to acquire the knowledge with regards in resistant to antifungal agents. Most commonly used antifungal susceptibility testing methods includes macro and micro broth dilution, but these methods are comparatively difficult to perform. Hence the need of simpler methods has to be established to solve such problems. Agar based disc diffusion sensitivity testing (ABDD) method for dermatophytes is simple, cost effective and does not require any specialized instrument and E test method can also help in determining the MIC of antifungal agents. This E-test method is a good alternative option for broth dilution[6]. Hence this current study was aimed to identify and evaluate the sensitivity patterns of dermatophytes.

Materials and Methods

All the clinical samples (Skin, Hair, Nail) that were received in the Department of Microbiology with the clinical suspicions were studied. All clinical suspicions that were processed for direct microscopic examination for detection of fungal filaments using different concentrations of potassium hydroxide (KOH 10% for skin scales, 20% for hair stumps & 40% for nail clippings). For culture, samples were inoculated onto Sabouraud dextrose agar containing cycloheximide, Potato dextrose agar and incubated at 25°C for 2-4 weeks. A special dermatophyte test medium was used for rapid detection of dermatophytes based on color change in the medium due to alkaline metabolites produced by the organism. Isolated fungi were identified based on macroscopic appearance (growth, texture, pigmentation etc.), &microscopic appearance of the fungi. Lacto phenol cotton blue (LPCB) mount technique was done to demonstrate fungal hyphae, conidia and spores. Antifungal sensitivity testing was performed by using disc diffusion method for three antifungal agents and E-test was performed for 4 antifungal agents according to the manufacturers protocol. Brief description of ABDD and E-test is as follows:

Anti-fungal susceptibility

Inoculum preparation: Inoculum was prepared according to standard protocol, A loopful of fungal growth was picked up from the potato dextrose agar and emulsified the colonies into 0.9% sterile saline and mix the broth by using vortex mixer or by gently shaking the tubes for 20-30 seconds. Then the prepared inoculum should be adjusted to 0.5 McFarland's standard.

Disc diffusion method: Agar based disc diffusion method was performed according to NCCLS guidelines. Three antifungal agents (Terbinafine 10µg, Fluconazole 25µg & Itraconazole 10µg) were used to test the isolated dermatophytes. Modified Sabouraud dextrose agar was used and the plates were lawn cultured by using sterile cotton swabs, After the lawn cultured plates were kept for drying before placing the antifungal discs. Once discs were placed, plates were incubated at 25°C for 48-72 hours. The inhibition zone diameter was measured by using zone measuring

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

scale.

E-test method: All the isolates were tested with four antifungal agents (Griseofulvin 0.02-32mcg/ml, Terbinafine0.02-32mcg/ml, Fluconazole 0.016-256mcg/ml & Itraconazole 0.02-32mcg/ml) by E-test method as per standard protocol. The inoculum suspension was lawn cultured onto modified Sabourauds dextrose agar and E-strips were placed at the center. The plates were incubated at 25°C for 48-72 hours. The MIC was noted at the point where growth was completely inhibited.

Results

Of 180 clinical suspicions that were received in the department of Microbiology for diagnosis of Dermatophytosis were included, of which 107 (59.4%) samples were collected from males and 73 (40.6%) were obtained from females. Out of 180 clinical specimens, 108(60%) were skin scrapings, 54(30%) nail clippings and 18(10%) were hair stumps. All the specimens were processed for KOH microscopy and inoculated onto Sabouraud dextrose agar containing cycloheximide, Dermatophyte test medium and Potato dextrose agar for isolation. Among 180 samples, 96 showed the presence of septate fungal hyphae suggestive of dermatophytosis and also yielded the growth of dermatophytes. 10 samples were only positive through microscopic examination but culture yielded no growth of dermatophytes and remaining 74 samples were both Microscopy and culture negative. **(Table 1)**. Clinical presentation of the subjects was noted among which 60% of the subjects presented with tinea *corporis*, 30% with *Tinea ungums* and 10% of the subjects with Tinea *capitis.* Inoculation of clinical samples onto Sabouraud dextrose agar containing cycloheximide, Dermatophyte test medium and Potato dextrose agar culture media, 96 (53.3%) specimens yielded the growth of Dermatophytes**(Table 2)**. 45 specimens yielded growth of Trichophyton rubrum, followed by 29 were Trichophyton mentagrophytes, 8 were Trichophyton tonsurans, 3 were Trichophyton verrucousum, 2 were Trichophytonviolaceum and remaining 5 yielded growth of Microsporum among which 3 were Microsporumgypseum and 2 were Microsporumaudonii**(Table 3)**. All the clinical isolates were tested for In-vitro antifungal sensitivity testing by two-methods disc diffusion and E-test and the results were compared. Of 96 isolates, 86.4% of the isolates were sensitive to all antifungal agents by E-test and 83.3% of the isolates were sensitive by disc diffusion method. 12 isolates were resistant to fluconazole, 9 were resistant to terbinafine, 4 were resistant to Itraconazole and 2 were resistant to Griseofulvin through E-test **(Table 4& Graph 1)**. Through disc diffusion, 11 isolates were resistant and 5 were intermediate to fluconazole, 7 were resistant and 2 were intermediate to terbinafine and 5 isolates were resistant to itraconazole **(Table 5& Graph 2).**

Discussions & Conclusion

Antifungal susceptibility testing of dermatophytes is considered as one of the advancements in medical mycology. The performance, specificity & sensitivity of antifungal susceptibility testing have shown good progress in the diagnosis of dermatophytosis[2]. Agar based disc diffusion method is used to determine the activity of antifungal agents against dermatophytosis. This method can also be used in day-to-day life for practical as wells as routine diagnostic purpose. Macro-broth and micro-dilution tests can be used to determine the MIC of antifungal agents against dermatophytes, but these assays are quite costly and require a special equipment's, media, micro plates and microtips[7]. E-test is comparatively good optional method to detect the minimum

inhibitory concentration values of various antifungal agents. E-test method can be used for routine diagnosis in the laboratory and can also help in the assessment of drug resistance.

The present study emphasized the importance of isolation, identification and antifungal susceptibility testing of dermatophytes which plays a major role in the diagnosis and management of dermatophytosis. One of the main objective of our study is to compare the rate of isolation and identification of dermatophytes using Dermatophyte test medium and SDAc. The total isolation rate of dermatophytes in our study is 53%. When compared with Dermatophyte test medium and SDAc, Dermatophyte test medium had isolation rate of 53% whereas SDAc had 48%. Both Dermatophyte test medium and SDAc showed significant rate in isolation but Dermatophyte test medium had slight higher rate of isolation when compared to SDAc, Hence Dermatophyte test medium can be recommended to use as screening medium for primary isolation and detection of dermatophytes.

In this study Trichophyton rubrum 45 (46.8%) and Trichophytonmentagrophytes 29 (30.2%)were the most predominant causative agents among dermatophytes followed by Trichophyton tonsurans 8 (8.3%). The similar results were mentioned by Barros et.al in their study Trichophytonrubrum (41%) is the most predominant causative agent followed by Trichophytonmentagrophytes (26%). We also tried to compare the MICs of E-strip method and IZDs of disc diffusion method for evaluation of sensitivity patterns of dermatophytes. Our study revealed 12% of the isolates showed resistant to Fluconazole, 9% to Terbinafine and 4% to Itraconazole by E-test method. Agar based disc diffusion method showed 11% of the isolates were resistant to Fluconazole, 7% to Terbinafine, followed by 5% to Itraconazole. Similar findings was shown by Howyda.M.Ebrahim et.al in their study 19% of the isolates were shown resistant to fluconazole and 13% were resistant terbinafine.

These antifungal susceptibility testing data can help to explain the promising results obtained for the treatment of dermatophytosis with this antifungal agents. Routine usage of antifungal susceptibility testing plays an important role in choosing the appropriate antifungal agent for the treatment. This study would give a discernment in drug resistance among dermatophytes.

Conflicts of interest

No potential conflict of interest relevant to this article was reported

References

[1] VanathiSabtharishi, Radhika Katragadda, Thyagarajan Ravinder, 2017. “A study on the antifungal susceptibility pattern of Dermatophytes isolated in a tertiary care hospital”. International journal of Bioassays.

[2] Mona fattouh Mohamed Shalaby, Asmaa Nasr-El-din. Mohammed Abu El-Hamd. Isolation,2016. “Identification and invitro antifungal susceptibility testing of Dermatophytes from clinical samples at Sohag university hospital in Egypt”. Electronic Physician (ISSN: 2008-5842).

[3] SowmyaN, Appalaraju, Srinivas Surendran P, 2015. “Antifungal susceptibility testing for Dermatophytes isolated from clinical samples by broth dilution method in a tertiary care hospital”. Journal of Medical Research.

[4] Howyda M. Ebrahim, Ahmed M. asaad, Ahmed Amer, 2015. “Antifungal Susceptibility

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

Patterns of Dermatophytes Clinical Isolates from Dermatophytosis Patients before and After Therapy". Eygptian journal of Medical Microbiology.

[5] Environ C.O. Agbulu, Iwodi, Onekutu, 2015. "In vitro Susceptibility Test of Some Antifungal Drugs on Selected. Dermatophytes and Yeasts Isolated from Patients Attending Hospitals in Makurdi". Microbiology Journal 9-16, DOI: 10.3923.

[6] R.K.Agarwal, S.Gupta, G.Mittal, F. Khan, S.Roy, A. Argawal, 2015. "Antifungal susceptibility testing of Dermatophytes by agar diffusion method" Int.J.Curr.Microbiol.App, sciences.

[7] TroChauVan, Khuong Ho Thi Ngoc, Thuong Nguyen Van, Khang Tran Hau, 2019. "Antifungal Susceptibility of Dermatophytes Isolated from Cutaneous Fungal Infections". Open Access Maced J Med Sci. 2019 Jan 30; 7(2):247-249.

[8] Shivaprakash M Rudramurthy, Dipika Shaw, 2019. "Overview and Update on the Laboratory Diagnosis of Dermatophytosis". Clinical Dermatology Review Published by Wolters Kluwer - Medknow. IP: 10.10.10.40.

[9] A. Anupama, 2017. "Isolation and Identification of Dermatophytes from Clinical Samples". IJCMAS.611.152.

Table 1: showing results of direct microscopy and culture

Total samples (180)	KOH Positive	KOH Negative	Total
Culture positive	90	6	96
Culture negative	10	74	84

Table 2: showing comparison between SDA ac and DTM in primary isolation of Dermatophytes

Total number of culture positive	96
Culture positive by SDA ac	87 (90.6%)
Culture positive by DTM	100 (100%)

Table 3: Showing Dermatophytes strains in relation to localization

Dermatophytes	No	*T.corporis*	*T.unguium*	*T.capitis*	*T.cruris*	*T.incognito*	*T.facei*	*T.pedis*
T.rubrum	45	36	-	-	5	3	1	-
T.menatgrophytes	29	12	6	3	2	1	1	4
T.tonsurans	12	2	-	10	-	-	-	-
T.verrucossum	3	2	-	1	-	-	-	-
T.violaceum	2	2	-	-	-	-	-	-
M.gypseum	3	2	-	1	-	-	-	-

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

M.audoinii	2	1	-	1	-	-	-	-
Total	96	57	6	16	7	4	2	4

Graph 1: Showing the E- test antifungal sensitivity patterns of 96 isolates

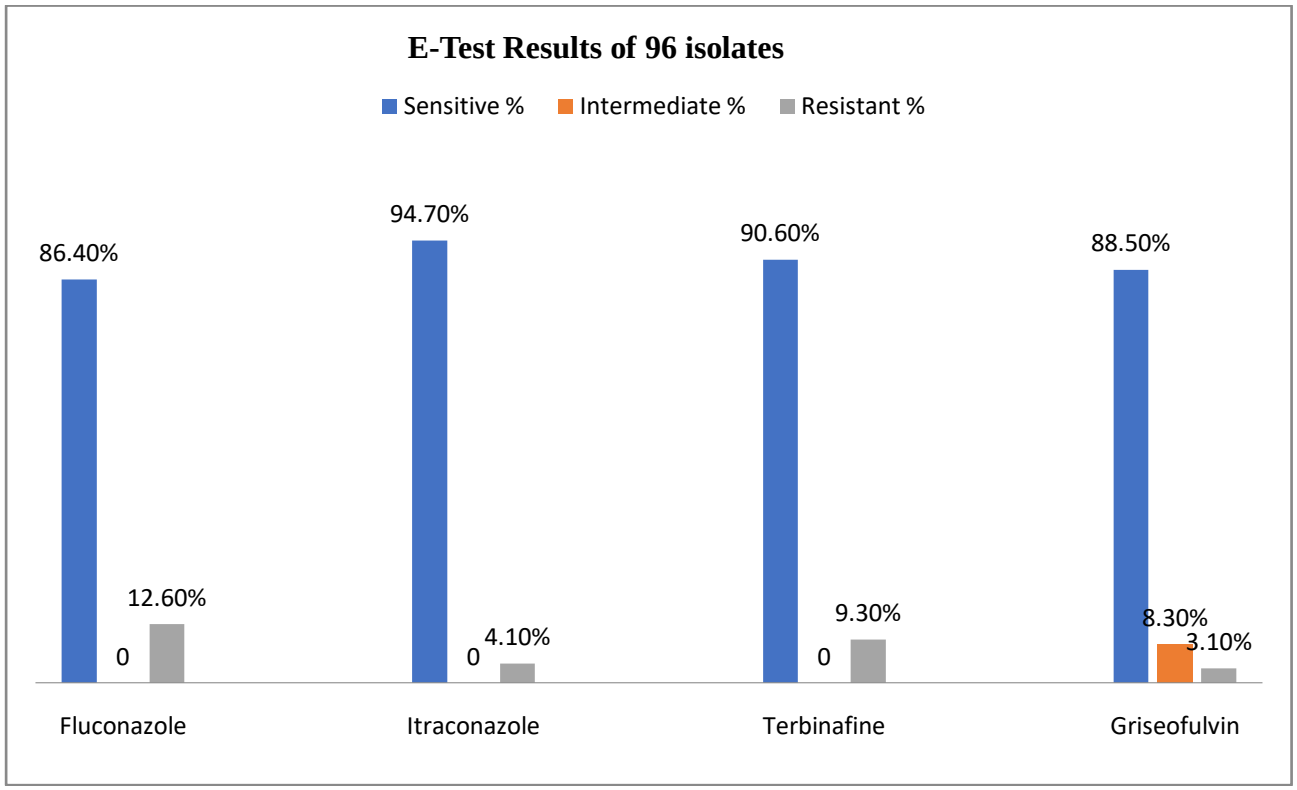


Table 4: Showing antifungal susceptibility patterns of Dermatophytes by using E-test method

Isolates (96)	Sensitive (%) (mcg/ml)	Intermediate (%) (mcg/ml)	Resistant (%) (mcg/ml)
T.rubrum(45)			
1. FLUCONAZOLE-(0.016-256µgm)-	38(84.4)	1(2.2)	6(13.3)
2. ITRACONAZOLE-(0.002-32µgm)-	41(91.1)	0	4(8.8)
3. TERBINAFINE-(0.002-32µgm)-	41(91.1)	0	4(8.8)
4. GRISEOFULVIN-(0.002-32µgm)-	36(80)	7(15.5)	2(4.4)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

T.mentagrophytes(29)			
1. FLUCONAZOLE-(0.016-256µgm)-	25(86.2)	0	4(13.7)
2. ITRACONAZOLE-(0.002-32µgm)-	29(100)	0	0
3. TERBINAFINE-(0.002-32µgm)-	25(86.2)	0	4(13.7)
4. GRISEOFULVIN-(0.002-32µgm)-	28(96.5)	0	1(3.4)
T.tonsurans(12)			
1. FLUCONAZOLE-(0.016-256µgm)-	10(83.33)	0	2(16.66)
2. ITRACONAZOLE-(0.002-32µgm)-	11(91.66)	0	1(8.33)
3. TERBINAFINE-(0.002-32µgm)-	11(91.66)	0	1(8.33)
4. GRISEOFULVIN-(0.002-32µgm)-	11(91.66)	1(8.33)	0
T.violaceum(2)			
1. FLUCONAZOLE-(0.016-256µgm)-	2(100)	0	0
2. ITRACONAZOLE-(0.002-32µgm)-	2(100)	0	0
3. TERBINAFINE-(0.002-32µgm)-	2(100)	0	0
4. GRISEOFULVIN-(0.002-32µgm)-	2(100)	0	0
T.verucossum(3)			
1. FLUCONAZOLE-(0.016-256µgm)-	3(100)	0	0
2. ITRACONAZOLE-(0.002-32µgm)-	3(100)	0	0
3. TERBINAFINE-(0.002-32µgm)-	3(100)	0	0
4. GRISEOFULVIN-(0.002-32µgm)-	3(100)	0	0
M.gypseum (3)			
1. FLUCONAZOLE-(0.016-256µgm)-	3(100)	0	0
2. ITRACONAZOLE-(0.002-32µgm)-	3(100)	0	0
3. TERBINAFINE-(0.002-32µgm)-	3(100)	0	0
4. GRISEOFULVIN-(0.002-32µgm)-	3(100)	0	0
M.audounii (2)			
1. FLUCONAZOLE-(0.016-256µgm)-	2(100)	0	0
2. ITRACONAZOLE-(0.002-32µgm)-	2(100)	0	0
3. TERBINAFINE-(0.002-32µgm)-	2(100)	0	0
4. GRISEOFULVIN-(0.002-32µgm)-	2(100)	0	0

Graph 2: Showing the ABD antifungal sensitivity patterns of 96 isolates

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

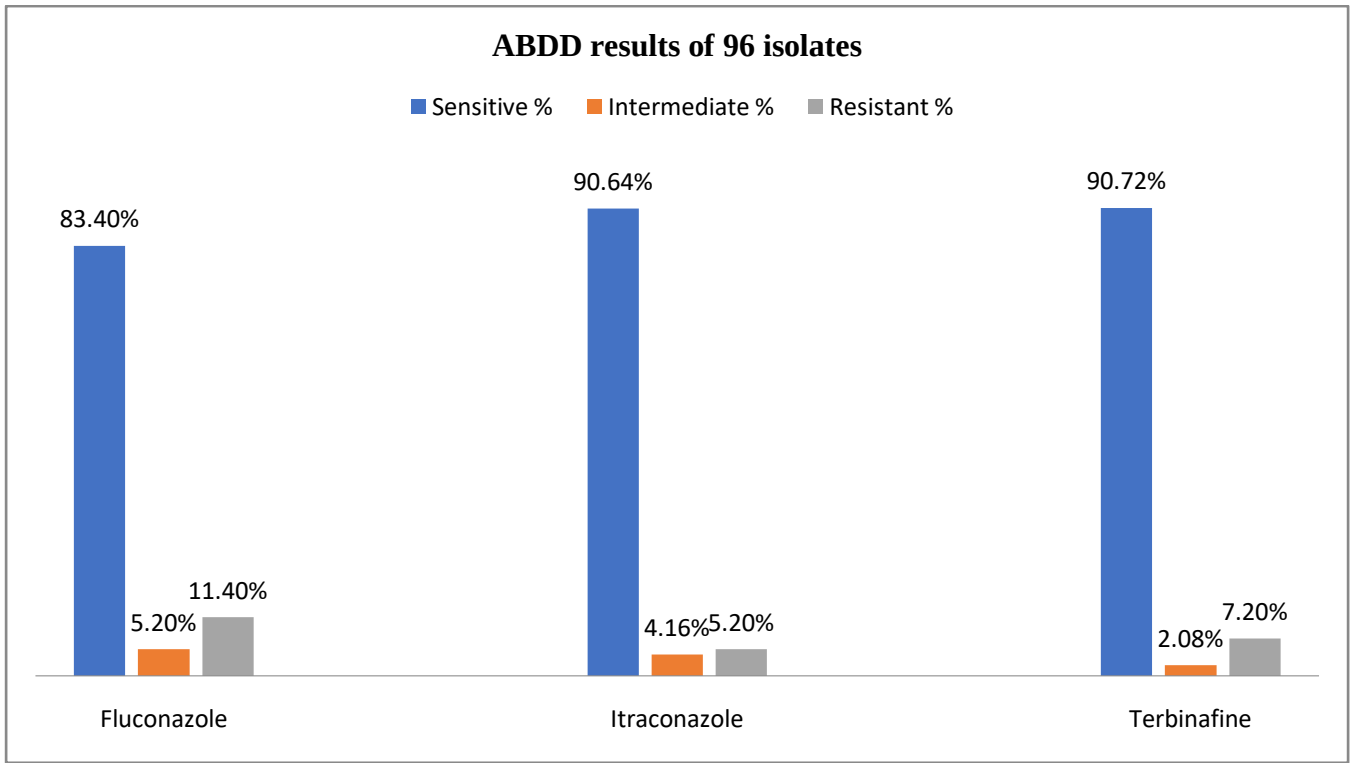


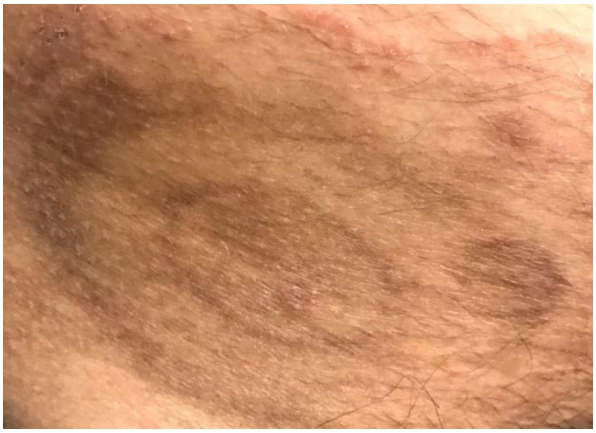
Table 5: Showing antifungal susceptibility patterns of Dermatophytes by using ABDD method.

Isolates (96)	Sensitive (%) (mcg/ml)	Intermediate (%) (mcg/ml)	Resistant (%) (mcg/ml)
T.rubrum(45)			
1. FLUCONAZOLE-(30 µgm)	37(82.2)	3(6.6)	5(11.1)
2. ITRACONAZOLE-(10 µgm)	39(86.6)	2(4.4)	4(8.8)
3. TERBINAFINE-(10 µgm)	41(91.1)	1(2.2)	3(6.6)
T.mentagrophytes(29)			
1. FLUCONAZOLE-(30 µgm)	23(79.3)	2(6.8)	4(13.7)
2. ITRACONAZOLE-(10 µgm)	21 (72.4)	2 (6.8)	0(0)
3. TERBINAFINE-(10 µgm)	25(55.5)	1(3.4)	3(10.3)
T.tonsurans(12)			
1. FLUCONAZOLE-(30 µgm)	10(83.3)	0	2(16.6)
2. ITRACONAZOLE-(10 µgm)	11 (91.66)	0	1(8.33)
3. TERBINAFINE-(10 µgm)	11(91.66)	0	1(8.33)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

T.violaceum (2)			
1. FLUCONAZOLE-(30 µgm)	2 (100)	0	0
2. ITRACONAZOLE-(10 µgm)	2 (100)	0	0
3. TERBINAFINE-(10 µgm)	2 (100)	0	0
T.verucossum (3)			
1. FLUCONAZOLE-(30 µgm)	3 (100)	0	0
2. ITRACONAZOLE-(10 µgm)	3 (100)	0	0
3. TERBINAFINE-(10 µgm)	3 (100)	0	0
M.gypseum (3)			
1. FLUCONAZOLE-(30 µgm)	3 (100)	0	0
2. ITRACONAZOLE-(10 µgm)	3 (100)	0	0
3. TERBINAFINE-(10 µgm)	3 (100)	0	0
M.audounii (2)			
1. FLUCONAZOLE-(30 µgm)	2 (100)	0	0
2. ITRACONAZOLE-(10 µgm)	2 (100)	0	0
3. TERBINAFINE-(10 µgm)	2 (100)	0	0

Figure 1: showing the clinical type of Tinea corporis (Ring worm) and Tinea capitis



Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 958 - 967
Received 08 November 2021; Accepted 15 December 2021.

Figure2: showing the septet fungal filaments on KOH wet mount

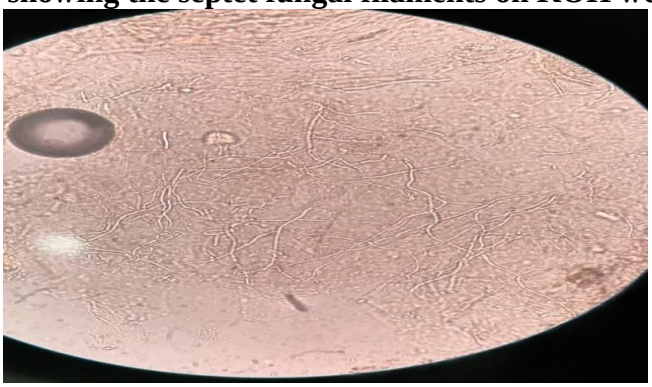


Figure3: showing the colonies on DTM medium

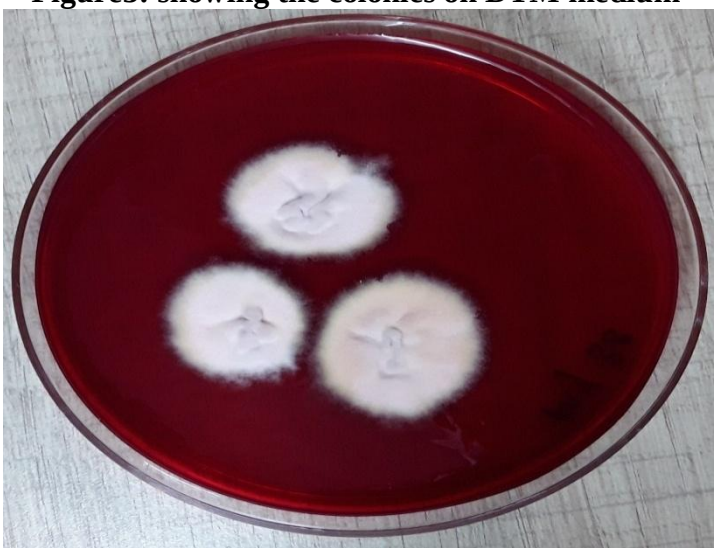


Figure5: Showing the sensitivity of antifungal agent by E-test method