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DERMATOPHYTE SUSCEPTIBILITIES TO ANTIMYCOTIC DRUGS BY DISC DIFFUSION METHOD

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ABSTRACT

Introduction: Superficial mycoses are common fungal infection of keratinized tissue. Now a day's several option of antifungal agent are available& clinician must now choose among them. Several report of treatment failure & relapse of dermatophytosis are there. So determination of in vitro antimycotic susceptibility is essential. In our study we evaluate antifungal activity of five antimycotic agents. **Material & methods:** Sixty seven dermatophyte were isolated from patient having dermatophytosis . Five antimycotic discs viz. nystatin, fluconazole ,ketoconazole ,itraconazole, clotrimazole were used by disc diffusion method for in vitro susceptibility testing against these isolates. **Result:** A total of sixty seven dermatophytes were isolated and identified. The isolates belong to two genera and four species as follows: *T. mentagrophytes 16*(23.8%),*T. rubrum* 38(56.7%), *T. violaceum* 7(10.4%), *M. canis* 6(7.58.9%). Regarding the data, it was revealed that nystatin and itraconazole were the most effective antimycotic drugs and clotrimazole had the poorest activity. **Conclusion:** The disc diffusion method is a simple, reliable, inexpensive and easily adaptable assay which is more practical, simple and easier in comparison to micro dilution method.

KEYWORDS: Dermatophyte , Antimycotic drugs, Disc diffusion method.

INTRODUCTION

Superficial mycoses are common worldwide. They are believed to affect 20% to 25% of the world's population, and the incidence continues to increase.[1] The incidence of fungal infections in the immuno- compromised population has increased greatly over the past two decades.[2] The most important risk factors for infection includes prolonged neutropenia, chronic administration of corticosteroids, the insertion of prosthetic devices, and tissue lesions due to prior infection or trauma. Therefore, the use of antimycotic agents has increased dramatically and new ones have also been developed, such as the triazoles and various amphotericin B lipid formulations.[3] The treatment of dermatophytosis is based on the use of topical and systemic antimycotic agents. While topical application of an antifungal is usually sufficient to eradicate the organism and to cure the majority of these infections, the most severe and chronic dermatophytosis, which includes some tinea unguium, scalp ringworm and skin lesions with folliculitis, often requires the administration of systemic treatment.[4]

With this proliferation of antifungal agents, therapeutically options have also increase and the clinician must now choose among them. The in vitro antimycotic susceptibility of the different pathogenic fungi can be a valuable guide for the clinicians. However, reliable antimycotic susceptibility testing is still poorly developed, especially for filamentous fungi.[5] Dilution tests are widely used in macro and micro-assays, but these methods are difficult to be used in most of the laboratories. Recently, studies were done to establish a simple method to solve this problem. The agar-based disk diffusion (DD) susceptibility method for dermatophytes is simple, inexpensive, and does not require specialized equipment. The disk diffusion method has a good correlation with the reference dilution assay.[6]

MATERIAL & METHODS

The dermatophytes were isolated from patients having superficial mycoses those attended Skin & V.D. Department of Dr. S.N. medical college & attached groups of hospitals, Jodhpur. Samples were taken from skin, hair and nail.

Isolates

These samples were inoculated on the slants of SDA with gentamycin (.05 mg /ml) and were incubated at 25-30^{0}C. These were examined weekly for four weeks and at the end of fourth week. KOH mount of samples and Slide culture preparation mounted in lacto phenol cotton blue were made for identification of etiological agents for studying colony characteristics

(colour of surface & reverse of colony, texture , topography) and microscopic morphological features like appearance and arrangement of the micro conidia, macro conidia and hyphae.

Preparation of the Inoculum

Inoculum is prepared from seven day culture growth on potato dextrose agar at 35^0C.on which subculture was done. Few isolated fungal colonies were mixed in 1ml of sterile 0.85% saline. The resulting mixture was allowed to settle for 3-5 minutes. The upper suspension was separated which contained mixture of conidia, sponogiospore & hyphal fragments. It was mixed for 15 seconds with a vortex and turbidity was adjusted with 0.5 Mac Farland standard that is $1x10^8$ CFU/ml (S.Khan et al 2006 haryana.[7] Suspension was further diluted with RPMI 1640 to final concentration of $1x10^4$ - 5 x 10^4 conidia/ml.

Inoculation on Agar Plate

It was done within 15 minutes of adjusting turbidity. Sterile cotton swab was dipped in inoculum rotated several times & pressed firmly against the side wall of tube above the fluid level which removes excess fluid. The entire dried agar surface was evenly streaked in three different directions swabbing near the rim of the plate as final step. Lid of the plate is left open for not more than 15 minutes to allow the agar surface dry. Then disc are dispensed and pressed down to ensure complete contact with agar. Discs were placed 24 mm apart from center to center .Plates were incubated with in 15 min after discs had been dispensed. Plates were incubated at 35 ˚C for 5-7days.

The following Antimycotic discs with mentioned disc concentrations were used.

Antimycotic Discs	Disc Contents	Diameter of Zone of Inhibition in mm
Nystatin	50 mcg	19-23
Fluconazole	10mcg	22-33
Ketoconazole	10mcg	14-19
Itraconazole	10mcg	11-18
clotrimazole	10mcg	16-30

(C.parapsilosis ATCC 22019 was used as control strain)

Interpretation of results

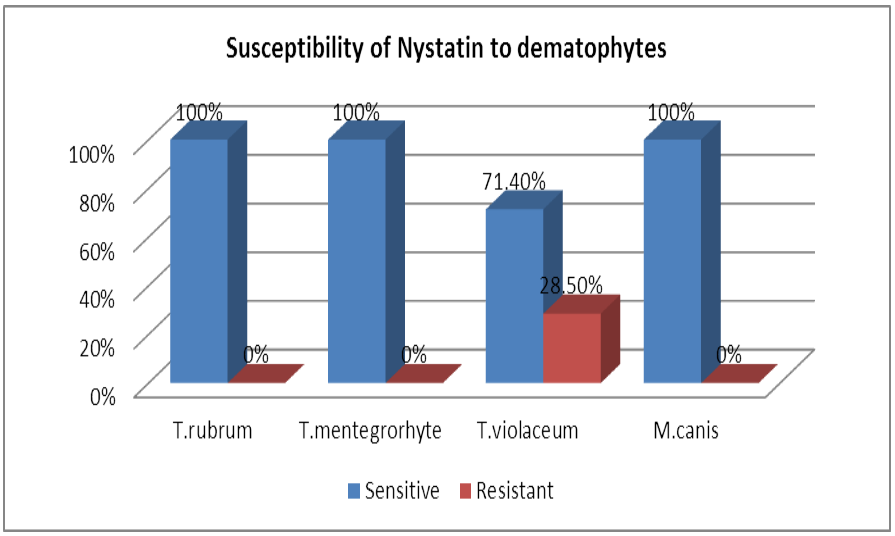
Results were read as per zone of diameter mentioned. Zone of inhibition was measured with ruler at the point of maximum reduction of growth. Pin point colonies at zone edge and large colonies within a zone were ignored.

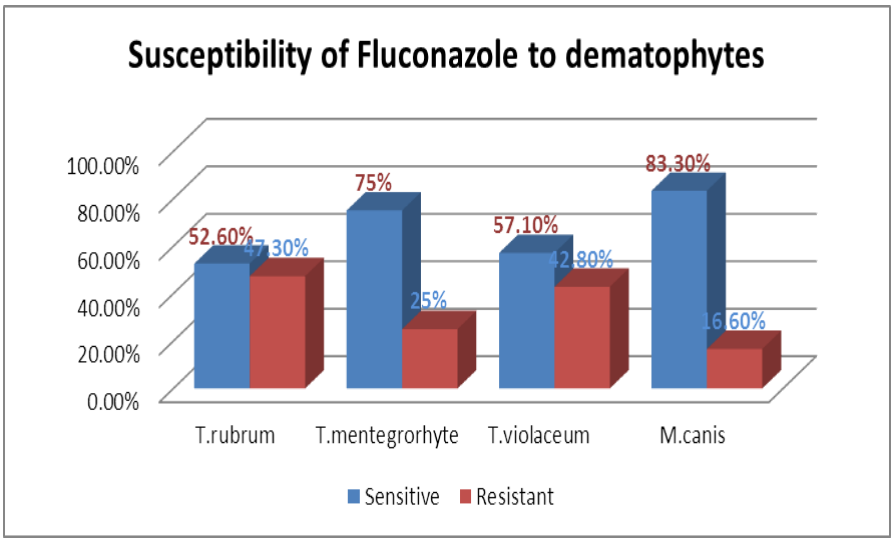
RESULT

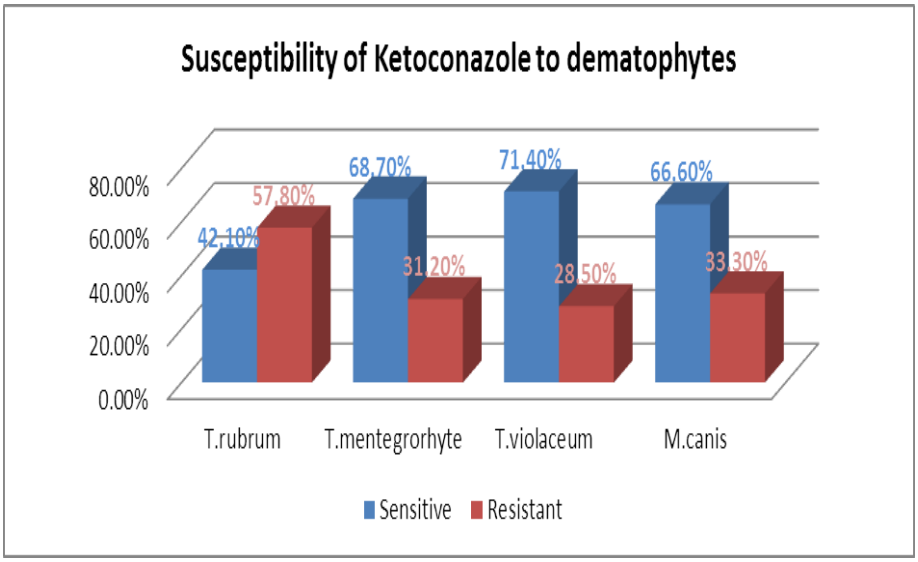
A total of sixty seven dermatophytes were isolated and identified. The isolates belong two genera and four species as follows: *T. mentagrophytes 16*(23.8%),*T. rubrum* 38(56.7%),*T.violaceum* 7(10.4%), *M. canis* 6(7.58.9%).

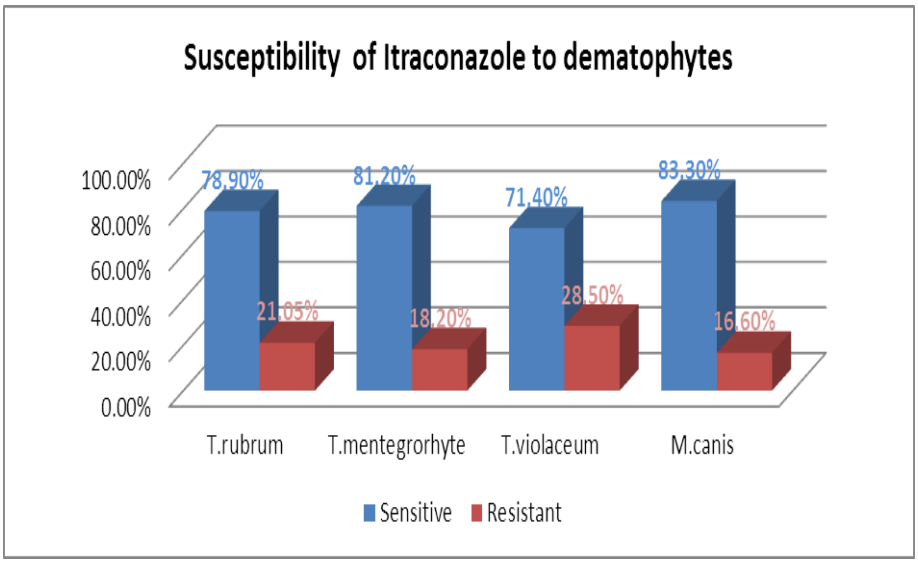
Effectivity of various antimycotic drugs against different species of dermtophytes

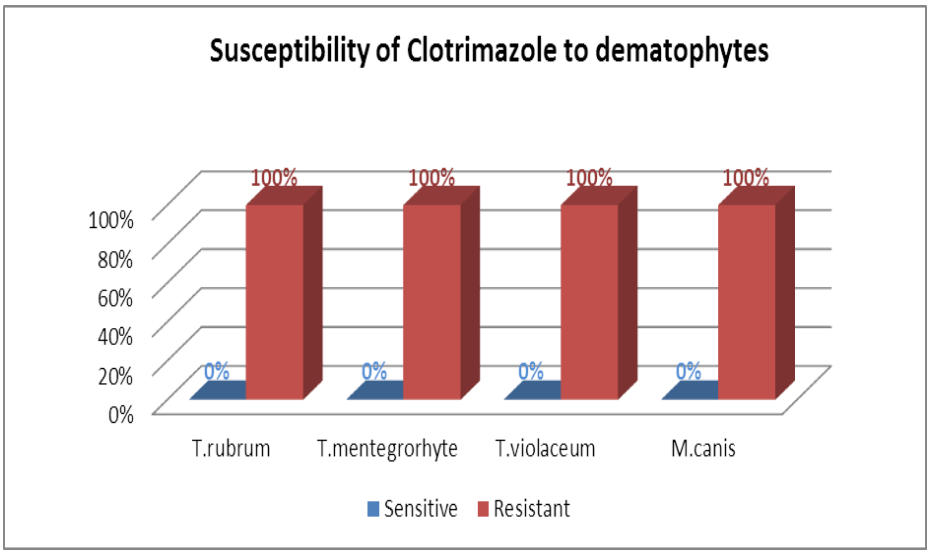
Isolates	Nystatin		Fluconazole		Ketoconzole		Itraconzole		Clotrimazole	
	S	R	S	R	S	R	S	R	S	R
T.rubrum	100%	0%	52.60%	47.30%	42.10%	57.80%	78.90%	21.05%	0%	100%
T.mentegrorhyte	100%	0%	75%	25%	68.70%	31.20%	81.20%	18.20%	0%	100%
T.violaceum	71.40%	28.50%	57.10%	42.80%	71.40%	28.50%	71.40%	28.50%	0%	100%
M.canis	100%	0%	83.30%	16.60%	66.60%	33.30%	83.30%	16.60%	0%	100%






Susceptibility of Ketoconazole to dematophytes
80.00%
60.00%
40.00%
20.00%
0.00%
42.10%
57.80%
68.70%
31.20%
71.40%
28.50%
66.60%
33.30%
T.rubrum
T.mentegrorhyte
T.violaceum
M.canis
Sensitive
Resistant

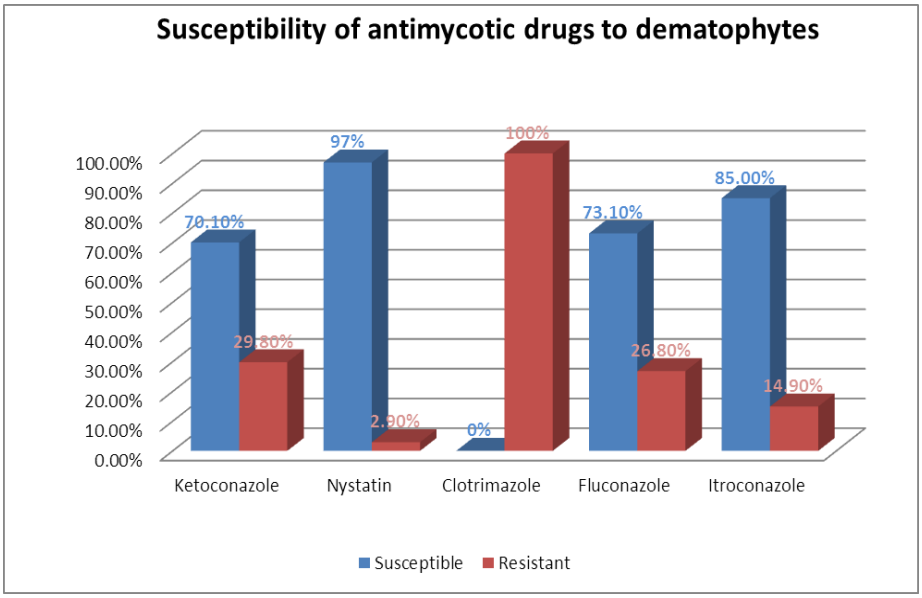

Susceptibility of Itraconazole to dematophytes
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
78.90%
21.05%
81.20%
18.20%
71.40%
28.50%
83.30%
16.60%
T.rubrum
T.mentegrorhyte
T.violaceum
M.canis
Sensitive
Resistant


Susceptibility of Clotrimazole to dematophytes
100%
80%
60%
40%
20%
0%
0%
100%
0%
100%
0%
100%
0%
100%
T.rubrum
T.mentegrorhyte
T.violaceum
M.canis
Sensitive
Resistant

Susceptibility of antimycotic drugs to dematophytes in total

Antifungal disc	Susceptible	Resistant
Ketoconazole	47 (70.1%)	20 (29.8%)
Nystatin	65(97%)	2 (2.9%)
Clotrimazole		67(100%)
Fluconazole	49 (73.1%)	18 (26.8%)
Itroconazole	57(85.0%)	10(14.9%)

The nystatin and itraconazole were showed maximum antimycotic sensitivity whereas clotrimazole was hardly effective against dermatophytes.



DISCUSSION

Antifungal susceptibility testing is a dynamic field of medical mycology.[8] In vitro antifungal susceptibility tests could help to optimize the therapy and to select an effective antifungal agent for dermatophytosis[9] Clinical and Laboratory Standards Institute (CLSI, formerly 'National Committee for Clinical Laboratory Standards', NCCLS) published (M38-A) document for antimycotic sensitivity by micro dilution method of filamentous fungi.[10] In May 2010 published a guide line (M-51A) for disc diffusion method of anti fungal susceptibility for mold other then dermatophytes. Guidelines of disc diffusion method for dermtophyte is not available by CLSI. Only standarad method available for anti-mycotic sensitivity for mould is micro dilution method. In our study we used disc diffusion method for antifungal susceptibility testing of dermatophyte isolates. In our study, clotrimazole was found least effective although it is oldest antifungal agent which is used topically against dermatophytes. It is not suitable for long term treatment in severe infections of hair and nail

for which systemic therapy is needed. Both nystatin and itraconazole were found most effective as comparable to the study by Siqueir.[11] It is perhaps because itraconazole is used parenterally so it would be difficult to be used as self medication.

Whereas clotrimazole is used injudiciously due to its topical use which might be responsible for its observed resistance. Fluconazole is intermediate between clotrimazole and itraconazole in regard to its anti mycotic sensitivity pattern. It is perhaps because fluconazole is used most commonly in clinical practice and might acquired mild degree of resistance in comparison to itraconazole and nystatin which showed highest degree of sensitivity.

CONCLUSION

The disc diffusion method is a simple, reliable, inexpensive and easily adaptable assay in comparison to other methods for anti-mycotic drug sensitivity including micro dilution method which is more expensive time consuming and require skill and specific media and equipment such as RPMI, MOPSs buffer, and micro plate trays.

REFERENCES

1. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses., 2008; 51(Suppl 4): 2-15.
2. Bodey, G. P. Azole antifungal agents. Clin. Infect. Dis. 1992; 14(Suppl.1): S161–S169
3. Wheat L. J. 1994. Fungal infections in the immuno compromised host, p.211–237. *In* R. H. Rubin and L. S. Young (ed.), Clinical approach to infection in the compromised host, vol. 3. Plenum Publishing Corporation, New York.
4. Comparison of In Vitro Activities of Voriconazole and Five Established Antifungal Agents against Different Species of Dermatophytes using a Broth Macrodilution method sofia perea, annette w. fothergill, deanna a. sutton, and michael g. rinaldi1, journal of clinical microbiology, 0095-1137/01/$04.0010 doi: 10.1128/jcm.39.1.385–388.2001 Jan. 2001; 39(1): 385–388
5. Comparison Study of Broth Macrodilution and Microdilution Antifungal Susceptibility Tests for the filamentous fungi pujol, j. guarro, c. llop, soler, and j. ferna´ndez ballart) antimicrobial agents and chemotherapy, Sept., 1996; 40(9): 2106–2110.
6. *In vitro* activity of six antifungal drugs against clinically important Dermatophytes Keyvan Pakshir, Leila Bahaedinie, Zahra Rezaei, Manuchehr Sodaifi, Kamiar Zomorodian, Jundishapur Journal of Microbiology, 2009; 2(4): 158-163 158 Jundishapur

Journal of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

7. Antifungal susceptibility testing method for resource constrained laboratories S Khan, S Singhal, T Mathur, DJ Upadhyay, A Rattan. Indian Journal of Medical Microbiology., 2006; 24(3): 171-176
8. Sevtap A. Current status of antifungal susceptibility testing methods. *Med Mycol*., 2007; 45: 569-87.
9. Georg LK and Camp LB. Routine nutritional tests for identification of dermatophytes. J. Bacteriol., 1956; 74: 113-21.
10. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, 2nd ed. Approved standard. Clinical and Laboratory Standards Institute, Wayne, PA. CLSI document., 2008; M38-A
11. Emerson Roberto Siqueira; Dermatophyte susceptibilities to antifungal azole agents tested in vitro by broth macro and microdilution methods, rev. inst. med. trop. S. Paulo, January-February, 2008; 50(1): 1-5.