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# Antifungal Sensitivity Pattern of the Fungi Isolated from Superficial Mycotic Infections in and around Chidambaram-South India

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**ABSTRACT**

Totally 120 numbers of the fungal isolates includes dermatophytes,non dermatophyte molds and candida has been tested for their susceptibility pattern to eight different antifungal drugs. Disc diffusion method has been followed to study their susceptibility pattern. The fungal isolates varied in their antifungal susceptibility pattern to the tested anti-fungal drugs. Some of the antifungal drugs which were not acted against candida isolates, found to shown their antifungal activity to dermatophytes and non dermatophytic molds. From our study we can conclude, that the necessities of doing anti-fungal sensitivity test for each fungi isolated from the fungal infections.In view of effective treatment, we can suggest that the periodical surveillance of the antifungal sensitivity of the fungal isolates which are primarily isolated from the clinical specimens, since they vary with their sensitivity pattern to the commercially available antifungal drugs.



## Introduction

The alarming threat of the microbial drug resistance put us in delicate situation. Every year, the worst condition of the antifungal resistance is documented by many authors at global level. In last two decades, the fungal infections includes, dermatophytosis has been documented at different geographical area increasingly (Elewski and Charif 1997, Espineletal 1995, Norris et al.,1999 and Weitzman et al., 1995).

For the appropriate therapy, in the treatment of fungal infections, the clinician should select the effective drug to treat the patients. For this it is essential to make a periodical surveillance check on the susceptibility pattern of the fungal pathogens, since they Varied in their susceptibility pattern to the currently available antifungals. In this study we have made an attempt to study the antifungal susceptibility pattern of the dermatophytes and nonderatophytic molds and yeast. The patients attended the opd of our college hospital was the selective group, and included. All these study population is belonging to the villages located in and around Chidambaram.

## Materials and Methods

### Fungal isolates used in this study

We tested 120 isolates of dermatophytes and non dermatophytes originally isolated from the different types of superficial mycosis. This includes each 10 numbers of *T. rubrum,T. mentagrophytes,E. floccosum,M. canis, M. gypseum, Candida albicans, C. krusei, C. glabrata , Fusarium,A. fumigatus, A. flavus,* and *Penicillum*.All these fungal strains were cultured/isolated from the clinical specimens collected from the suspected cases of different types of superficial mycotic infections.

### Antifungal Agents Tested

Totally 8 different antifungal drugs currently available in Tamil Nadu,India (Himedia - Company – Mumbai, India) has been included in this study. The antifungal discs, Clotrimazole, Fluconazole, Ketaconazloe, Nystatin. Amphotericin-B,Itraconazole, Miconazole and Voriconazole were purchased and used.

### Fungal Culture Media Used

For the non dermatophytic molds and for candida, SDA with corn meal agar and dextrose(50%+50% and 20% dextrose)was used as culture medium. While for the dermatophytes,the special and selective media (dermatophytic agar Himedia-Mumbai) was used to culture both the clinical specimens, as well as for the fungal subcultures for sporulation .

### Invitro Antifungal Susceptibility Testing

### Standardization of Fungal Inoculum

The method of Norris et al., (1999) was used with slight modifications.T he fungus stock cultures were sub cultured on Sabourauds dextrose agar mixed with cornmeal agar and 20% dextrose, (50%+50% added with 20% dextrose).The inoculated agar slants were incubated at 37$^{o}$C for candida and non dermatophytic molds. For dermatophytes, they were inoculated on dermatophyte agar added with 50% cornmeal agar, and incubated at 25$^{O}$C in B.O.D incubator. The non dermatophytic fungi were incubated for four days while the dermatophytes were incubated for 5 to7 days. 85% sterile normal saline (15ml) was added to the culture slants and the tubes were placed in the VDRL shaker for 5 minutes at 180 rotation per minute. After, the fungal suspension was centrifuged at 10000 RPM and the supernatant fluid was discarded. The final solution at bottom of the test tube was adjusted to 3ml volume with normal saline.

The fungal solution was subjected to conidial evaluation under light microscope at 45x magnification. Presence of 10 and above, fungal spores per high power field was considered as satisfactory. The over crowded fungal spores were diluted further by adding the sterile normal saline, till we get the

desired fungal spore inoculum. This was considered as the standardized fungal spore inoculum for the antifungal testing.

In case of candida species yeast inoculum was prepared by mixing candida culture matching to Mac Farland opacity no. 0.5 in saline(Equals to $10^6$ cells/ml) this standardized candida inoculum was used in the antifungal testing.

**Disc Diffusion Method**

The Kirby Bauer disc diffusion method was used to study the antifungal susceptibility check for the selected fungal isolates. NCCLS guidelines was followed in the antifungal testing. Briefly the standardized fungal inoculum prepared with fresh sub culture (10micro liters) was swabbed on the respective media. The eight different antifungal discs were placed on the already inoculated agar media as per the NCCLS guidelines. The plates were incubated at $37^o$ C and $25^o$C for the non dermatophytes and dermatophytes respectively. The incubation period was extended up to 72 hours for non dermatophytes and for dermatophytes 4 to 5 days. the zone of inhibition around each disc was noted and recorded.

**Results and Discussion**

The superficial mycotic infections creating significant negative impacts on the patients and lead to social stigma. The appearance of the fungal lesion on the visual/external areas of the human/patients body put the individuals in bad psychological condition and even they tend to away from their close relatives.

This social problem requires the attention of the national authorities and policy makers, not only to overcome the problem but also to render their co-operation and encourage ment to be extended to the scientific/medical research sources to obtain the effective toxic less solution to treat the infected cases.

In view of finding the solution for the treatment of emerging fungal infections, it is must for us to find the current status of the fungal population involved in the particular superficial mycotic infections. Accordingly it is felt essential to study their susceptibility pattern to the currently available antifungal drugs, since each fungal strains exhibits differences in their susceptibility pattern to each antifungal drugs.

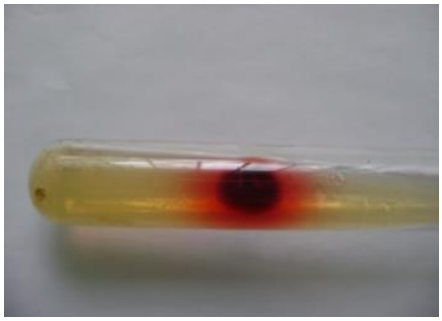

**Fig. 1. *T. rubrum.***

**Note:**Red pigment

**Fig. 2. Dermatophytic skin lesion.**

**Note:**Typical round skin lesions

**Fig. 3. *M. canis.***

**Note:** Resistant to six antifungals - MDR strain

**Fig. 4. *T. mentegrophytes.***

**Note:** Resistant to four antifungals

**Fig. 5. *A. flavus.***

**Note:** The strain sensitive to all eight antifungals

**Fig. 6. *C. albicans.***

**Note:** The strain sensitive to single antifungal drug

**Fig. 7. *C. krusei.***

**Note:** The strain sensitive to all antifungals

**Table 1. Antifungal Test Results of the fungal isolates (ZOI – mm in dia).**

| Sl. No. | Fungal Isolates (N = 10) | Antifungal Drugs Tested | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Clo | Flu | Ket | Nys | Am-B | Mic | Itr | Vori |
| I | **Non-Dermatophytes** | | | | | | | | |
| 1. | *Candida albicans* | 20 | 18 | 10 | 15 | 8 | 28 | 22 | 30 |
| 2. | *C. krusei* | 22 | 25 | 8 | 10 | 5 | 20 | 22 | 28 |
| 3. | *C. glabrata* | 10 | 15 | 8 | 11 | 7 | 25 | 15 | 28 |
| 4. | *Fusarium sp* | 15 | 12 | 3 | 5 | 10 | 15 | 18 | 30 |
| 5. | *Penicillum sp* | 8 | 15 | 12 | 10 | 15 | 18 | 22 | 30 |
| 6. | *Aspergillus flavus* | 9 | 12 | 15 | 18 | 21 | 28 | 25 | 33 |
| 7. | *A. fumigatus* | 5 | 8 | 12 | 15 | 18 | 23 | 28 | 35 |
| II | **Dermatophytes** | | | | | | | | |
| 8. | *T. mentagrophytes* | 5 | 3 | 8 | 10 | 10 | 15 | 18 | 32 |
| 9. | *T. rubrum* | 4 | 3 | 8 | 15 | 18 | 18 | 15 | 30 |
| 10. | *E. floccosum* | 15 | 18 | 22 | 25 | 32 | 30 | 33 | 28 |
| 11. | *M. canis* | 8 | 12 | 10 | 11 | 15 | 18 | 9 | 12 |
| 12. | *M. gypseum* | 10 | 15 | 8 | 15 | 12 | 22 | 28 | 35 |

**Note:** This table representing the maximum measure of the zone of inhibition expressed by the respective fungal species.

**Table 2. Antifungal Resistance Pattern of the fungal isolates (Overall Results in %).**

| Sl. No. | Fungal Isolates (N = 10) | Antifungal Drugs Tested | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Clo | Flu | Ket | Nys | Am-B | Mic | Itr | Vori |
| I | **Non-Dermatophytes** | | | | | | | | |
| 1. | *Candida albicans* | 60 | 90 | 90 | 20 | 80 | 10 | 10 | 0 |
| 2. | *C. krusei* | 80 | 70 | 80 | 50 | 80 | 0 | 0 | 0 |
| 3. | *C. glabrata* | 30 | 20 | 60 | 20 | 70 | 0 | 0 | 0 |
| 4. | *Fusarium* | 90 | 90 | 90 | 40 | 80 | 10 | 10 | 0 |
| 5. | *Penicillum* | 40 | 70 | 80 | 30 | 90 | 10 | 10 | 0 |
| 6. | *Aspergillus flavus* | 10 | 20 | 80 | 40 | 90 | 0 | 0 | 0 |
| 7. | *A. fumigatus* | 90 | 40 | 20 | 30 | 80 | 10 | 0 | 0 |
| II | **Dermatophytes** | | | | | | | | |
| 8. | *T. mentagrophytes* | 90 | 90 | 80 | 40 | 80 | 10 | 0 | 0 |
| 9. | *T. rubrum* | 90 | 90 | 70 | 60 | 80 | 10 | 10 | 0 |
| 10. | *E. floccosum* | 80 | 70 | 50 | 20 | 90 | 10 | 0 | 0 |
| 11. | *M. canis* | 90 | 90 | 100 | 60 | 100 | 10 | 0 | 0 |
| 12. | *M. gypseum* | 20 | 100 | 100 | 10 | 90 | 0 | 0 | 0 |

**Note:** This table representing the overall antifungal drug resistance recorded with the respective fungal isolates.

The antifungal drug resistance has been documented by many authors and they discussed about the dangerous conditions of the drug resistant infections (Elewski and Charif 1997, Espineletal 1995, Norris et al., 1999 and Weitzman et al., 1995). In our study, we could able to record the antifungal activity of the dermatophytic and non dermatophytic molds as well as the candida species, all these fungal strains were originally isolated from the different types of superficial mycotic infective cases.

When we tested all these fungal strains for antifungal susceptibility to eight different antifungal drugs, each fungal strain had shown their specific susceptibility patterns towards the tested drugs.at this junction, we would like to bring the attention of the physicians/dermatologists, those who are in the responsibility of treating the fungal infections. Since each fungal strains exhibiting differences in their susceptibility patterns, and even showing resistance to multiple antifungals instead of random antifungal treatment, the specific antifungal selection would bringout the effective cure. Hence we suggest to perform the fungal culture and antifungal testing as routine criteria to be carried out with each individuals when they come with the complains of the superficial mycotic infections or other types of fungal infections.

The poor conidia production by the fungi will hamper the determination of the susceptibility or resistance of the particular poor conidia producing fungi. So it is very much to ensure the conidia production. The valuable points to be followed while performing the antifungal testing of the dermatophytes were discussed by the authors Norris et al., 1999 and Weitzman and Summerbell 1995. Though they have explored and used such valuable methods in the optimization of antifungal susceptibility testing, in our study, we had followed certain innovative, feasible, tools/methods in the optimization of the fungal spores for the antifungal testing.

The Sabourauds Dextrose Agar with Corn meal agar and dextrose found to be the best medium to produce fungal spores of non dermatophytes and addition of corn meal agar and dextrose to the dermatophyte selective medium favored the production of spores of the dermatophytes, from this we suggest that these fungal culture medium for the sporulation of the fungi, especially when we are proceeding with the antifungal susceptibility testing with dermatophytes and non dermatophytic molds.

**Conclusion**

From our study results, it was confirmed that the fungal isolates isolated from different types of superficial mycotic infections exhibiting differences in their susceptibility to the currently available common antifungal drugs in view of improving the quality of patients life and to bring out the effective fast curing it is suggested that to follow fungal culture and sensitivity for each cases to ensure the specific sensitive antifungal drugs, to which the isolated fungi responds. We welcome future collaborative with interested author's research at molecular level study of this field of specialization.

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