Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 5
ISSN:2321–3647(online)

Antifungal Drug Sensitivity on Clinical Isolates From Dermatophytosis

Jyothi Padmaja.I[*1], Sirisha.T[1], Lakshmi.N[1], Harshitha.C[2], Bala Muralikrishna.P[1]
1. Department of Microbiology, Andhra Medical College, Visakhapatnam, India.
2. National Institute of Technology, Rourkela, Orissa, India.

ABSTRACT

The aim of the study is to test fungi isolated from cases of dermatophytosis against six antifungal drugs i.e., Amphotericin B(AP), Itraconazole (IT), Fluconazole (FLU), Clotrimazole(CC), Nystatin(NT) & Ketoconazole (KT). The isolated fungi are Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum, Candida albicans and Aspergillus niger. The study revealed that Clotrimazole and Ketoconazole were the most effective antifungals against dermatophytes, Nystatin for Candida albicans and Fluconazole for Aspergillus niger. The method used was disk diffusion method.

Keywords: Disk diffusion method, dermatophytes, antifungal agents.

*Corresponding Author Email: saidevic@gmail.com
Received 07 May 2014, Accepted 15 May 2014

Please cite this article in press as: Padmaja JI *et. al.*, Antifungal Drug Sensitivity on Clinical Isolates From Dermatophytosis. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Several factors have been attributed to the increase in fungal infections in the recent past. Most notable are organ transplantation, HIV/AIDS, cancer, diabetes, use of broad spectrum antibiotics and cytotoxic-chemotherapy.

The dermatophytes are closely related keratinophilic fungi which cause dermatophytosis. The incidence of dermatophyte infection has increased considerably during the past several decades[1]

Candida species are the most common opportunistic pathogens in compromised host. Candida albicans is the 5th most common cause of nosocomial blood stream infection[2]. It is the causal agent of opportunistic oral and genital infections and candidial onychomycosis and infection of nail plate[3].

Serious infections due to Aspergillus species and other filamentous fungi are emerging as prominent cause of infection, morbidity and mortality world wide[4,5].

Patients suffering from fungal infections receive a variety of antifungal agents during the course of their infection, classes of antifungal agents that are frequently used are Azoles[6] and polyenes[7,8].

MATERIALS AND METHODS:

Skin scrapings, nail and hair specimens were collected from patients with suspected dermatophytosis. Sample was obtained from patients attending the Dermatology outpatient department, King George Hospital of Andhra Medical College, Visakhapatnam (India). The affected area was thoroughly cleaned with 70% alcohol to remove the surface contaminants. Whatman no.1 filter paper was used for collecting specimens[9]. After disinfection with alcohol, skin lesions were scraped with blunt end of scalpel to collect epidermal scales. From the scalp hair was epilated with sterile forceps. Nail samples were collected using nail clip. 10% KOH solution was used for skin & hair and for nail scrapings 20% KOH was used. All preparations were examined under low power and confirmed under high power. Samples were also cultured on duplicate plates of Sabouraud Dextrose Agar (HiMedia) prepared according to the manufacturer's instructions and actidione & antibiotic were added. The plates were inoculated with finely divided pieces of each sample and incubated at 28^{0}C in BOD incubator (Remi) for recovery of dermatophytes or other fungi. The cultures were identified by their macroscopic and microscopic features[10,11,12]

Dermatophytes - T. rubrum colonies were white to fluffy, occasionally powdery to granular with diffuse wine red colored pigmentation on reverse. Microscopically showed Pyriform

microcondia borne slightly along the hyphae and Macrocondia which were pencil shaped. A negative urease test and hair perforation test further confirms T.r ubrum.

T.mentagrophytes colonies were creamy tan, powdery to granular, flat surface with buff colour. Microscopically abundant spherical microconidia were seen in grape like clusters and macroconidia were thin walled and cigar shaped. Sometimes spiral hyphae were seen, biochemical test like urease and in-vitro hair perforation test were positive[13].

Epidermophyton floccosum colonies grow rapidly within 3 to 5 days initially gray white then develop distinct characteristic khaki-green pigment when mature. Yellow white streamers of hyphae may be seen radiating from the center of the colony to periphery. The surface becomes granular on further maturity as conidia are produced and microconidia are never produced. Macrocondia are usually produced in profusion and are typically club-shaped, have three to five cells and thin, smooth walls. They often cluster in groups of three to four. Chlamydoconidia are typically present, particularly in older cultures.

Candida albicans colonies are cream coloured, glabrous, smooth and yeast like in appearance. Microscopically Gram positive budding yeast cells seen. It was identified as albicans by positive germ tube test within two hours in human serum at 37^{o}C and production of chlamydospores on corn meal gar at 20^{o}C.

Aspergillus niger showed wooly colonies, white to yellow at first and then turns to dark brown or jet black. Granular surface due to dense proliferation of black spores (condiospores). Microscopically it showed hyaline septate hyphae with black head and conidia, conidiophores of variable length, biseriate phialades covering entire vesicle.

Antifungal drugs - Amphotericin B disks(20 µg), Itraconazole disks (10µg), Fluconazole disks (10µg), Clotrimazole disks (10µg), Nystatin disks (100µg) & Ketoconazole disks (10µg) concentration were used in this study obtained from Himedia.

ANTIFUNGAL SENSITIVITY TESTING:

Preparation of Inoculum and Agar well diffusion Method –

Preparation of Inoculum and media on which the disks were placed and incubation period was different for Dermatophytes, Candida and Aspergillus. 21 days old grown culture of T. rubrum, T. mentagrophytes, E.floccosum were scraped with sterile scalpel and dissolved in sterile saline solution to make different dilutions (1/2). One of diluted suspensions was used as inoculum which had absorbance of 0.600 at 450nm determined spectroscopically (Electronics India)[14,15].

Antifungal screening was carried out using the agar well diffusion assay(CLSI,M-27A, M-28A)[16]. 20 ml of sterilized Sabouraud dextrose agar medium was poured into a 15 cm Petri

dishes in triplicates for each fungi. 20 µl of inoculum suspension of the T. rubrum, T. mentagrophytes, E.floccosum was distributed evenly over the surface and antifungal disks were placed respectively and plates kept in BOD at 27°C for 10 to 15 days and zones of diameter were noted by the help of zone scale reader (Hi Media).

For Candida the yeasts were grown on SDA for 24 Hrs, inoculum was prepared and adjusted to match the turbidity of 0.5 MC Farlands standard using spectrophotometer (EI) set at 530nm wave length. Sterile applicator swab was moistured in that cell suspension and used to inoculate the surface of Muller Hinton Agar plate (20ml) supplemented with 2% glucose and methylene blue (0.5µg/ml) and than antifungal disks were placed and incubated in BOD for 24 Hrs and observed for zones of inhibition. (CLSI, M44A)[17]

For Aspergillus, the fungi were sub cultured on potato dextrose agar one week before to testing and the inoculum suspensions were prepared to optical densities ranged from 0.09 to 0.11. The agar plates were inoculated with inoculum suspension with sterile swab and antifungal disks (HiMedia) were placed (CLSI, M51A)[18] and incubated for 48 hours and observed for zones of inhibition.

RESULTS AND DISCUSSION

Table - 1

	No of Strains	**Zone of inhibition in mm**					
Isolates	(n=30)	**AP**	**IT**	**FLU**	**CC**	**NT**	**KT**
T.rubrum	16	0	13	0	32	0	22
T.mentagrophytes	8	0	14	0	31	0	25
E.floccosum	2	0	16	0	33	0	23
C.albicans	2	15	18	35	21	29	32
A.niger	2	20	12	0	13	27	12

Espinel- Ingroff[19] suggested the zones of inhibition for filamentous fungi as
For Azoles sensitivity zones were Susceptible - when ≥ 17 mm diameter, Intermediate - in between 14 mm-16 mm diameter, Resistant - when ≤ 13 mm diameter
For Amphotericin B sensitivity zones were Susceptible - when ≥ 15 mm diameter, Intermediate - in between 13mm-14 mm diameter, Resistant - when ≤ 12mm.
Sara Carralhinho[20] suggested the zones of inhibition for candida as **For Nystatin** Susceptible - when ≥ when 15mm diameter, Intermediate - in between 10 mm -14mm diameter, **Resistant - when no zone**

In the present study susceptible as well as intermediate sensitive were taken as sensitive (≥13mm), and resistant when ≤ 12mm and Table-1 shows Trichophyton rubrum, Trichophyton

mentagrophytes, Epidermophyton floccosum showed highest zone of inhibition for CC & KT suggesting sensitivity towards those drugs while Candida albicans showed sensitivity to all the six drugs and highest zone of inhibition for FLU followed by KT. Aspergillus niger showed sensitivity to NYS followed by AP. (Table-1)

Sensitivity of T.rubrum, C.albicans, A.niger tested for antifungal drugs Amphotericin B(AP), Itraconazole (IT), Fluconazole (FLU), Clotrimazole(CC), Nystatin(NT) & Ketoconazole (KT)

Dermatophytosis, now can be treated by a variety of antifungal agents. Tinea unguium (infection of nails), Tinea capitis (infection of scalp) being of a more extensive involvement need systemic therapy, whereas some fungal infections can be tackled just by topical application.[21] Fast developing antifungal resistance is one of the reasons that explains occasional therapy failure. This necessitates the development of a simple standardized and reproducible in-vitro assay, for evaluating the resistance contemporarily encountered.[22] The three main areas where antifungal susceptibility tests are being used presently are a) epidemiological surveys, b)assessment of the degree of antifungal activity c) optimized antifungal therapy and clinical outcome prediction.[23] Against these resistant pathogenic fungi, for testing the antifungal susceptibility quite a few novel methods are developed.[23,24] About two decades ago (1983) NCCLS, presently CLSI developed numerous studies to evolve a standardized antifungal susceptibility assay. Though the dilution tests are used frequently in many micro & macro assays they are quite cumbersome for many a laboratory. To find a simpler alternative, number of studies were undertaken recently.[23,25,26] A simple and cost effective method obviating the use of specialized equipment is the agar based disk diffusion susceptibility method, which has a reasonably good correlation with the referred dilution assay.[21,27,28,29]

One of the challenging areas of the hour is the development and standardization of antifungal susceptibility testing. Presently the standard disk diffusion assay is a good model not only for the antifungal susceptibility testing but also characterizing some fungal genera apart from being

useful in routine mycological diagnostic services. This method is reproducible and also correlates positively with the reference method.[30,31]

In our study, Clotrimazole and Ketoconazole had large inhibition zones around the disks. Clotrimazole had the best activity against the isolates of Dermatophytes. It is one of the oldest anti fungal drugs formulated as a topical for use against dermatophytosis[32]. The other fungal drug used in the study includes Ketoconazole that showed good activity[32]. Fluconazole has poor activity on isolates tested for Dermatophytes and Aspergillus. There are many studies indicating that Fluconazole has less activity against the above fungi[33]. Our data is in agreement with those reports. For Aspergillus niger, Nystatin was proved to be the best by showing largest zone of inhibition and same was reported by Valerie[34]. Candida albicans showed largest zone of inhibition for Fluconazole our studies are in comparison with Ball[35].

CONCLUSION:

Finally the study concludes that Clotrimazole showed significant sensitivity towards Dermatophytes, Fluconazole for Candida and Nystatin for Aspergillus. Identification of the causative fungus helps in administering the appropriate antifungal agent.

ACKNOWLEDGEMENT:

The authors are very much thankful to Department of Microbiology, Andhra Medical College, Visakhapatnam for providing required facilities.

REFERENCES:

1. Jessup CJ, Warner J, Isham N, Hassan I, Gratimoum MA. Antifungal susceptibility testing of dermatophytes establishing a medium for inducing conidial growth and evaluation of susceptibility of clinical isolates. J. Chem. Microbiol 2000; 38(1): 341-344
2. Edwards JE. Invasive Candida infections- evolution of a fungal pathogen. New England Journal of Medicine 1991; 324..1060 -1062
3. Ryan K J, Ray C G. Sherri's Medical Microbiology. ivth Ed Mc Graw Hill 2004: ISBN 0-8385-8529-9
4. Groll Ah Shah PM, Metzel C, Schineider M, Just – Nuebling F, Huebner K. Trends in the post-Mortem epidemiology of invasive fungal infection at a University Hospital. Journal of infections 1996: 33::23-32
5. Kim DM, Suh M K, Ha GY, Sohng S H. Finger nail Onychomycosis due to Aspergillus niger, Ann.Dermatol 2012; 24(4): 459-463

6. Espinel –Ingroff, A, Arthington- Skaggs B, Iqbal N, Ellis D, Pfaller M A, Messer S, Rinaldi M, Fother gill A, Gibbs D L, and Wang A. Multicellular evaluation of a New Disc Agar Diffusion Method for Susceptibility Testing of Filamentous fungi with Vericonazole, Posaconazole, Itraconazole, Amphotericin B and Caspofungin. J. Clin. Microbiol 2007; 45(6) : 1811-1820
7. Andrew Wein Stein, Brain Berman. Topical Treatment of common superficial Tinea infection. Am. Fam. Physician 2002; 65 (10): 2095-2103
8. Monica cheesbroush, Medical Laboratory manual for tropical countries. ELBS/Tropical Health Technolgy, Butter worths, United kingdom, 1989; 2: pp104.
9. Elmer W, Koneman, Stphen D.Allen, Willam M Janda, Paul C, Schereckeh Berger, Washington C. Winn. Color Atlas and text book of Diagnostic Microbiology 5th Edition Philadelphia William & Wilkins Publications 1997: 996-997.
10. Willam G, Merz and Roderick J. Hay. Microbiology and Microbial infections Medical Mycology 10th Edition Topley & Wilsons .2005: ISBN 034088567.
11. Jagadish Chander, Text book of Medical Mycology 2nd Edition New Delhi, Mehta Publishers 2002: 376.
12. Mackie and Mac cartney. *Practical Medical Microbiology*, Churchill Livingstone Publisher (Elsevier); 1996: 14: pp141.
13. Natarajan V, Venegopal PV, Menon T. Effect of Azadiracta indica (neem) on the growth pattern of dermatophytes. *Ind J Med Micro* 2003; **21**(2): 98-101.
14. Balakumar S, Rajan S , Thirunalasundari T, Jeeva S Antifungal activity of Aegle marmelos (L.) Correa (Rutaceae) leaf extract on dermatophytes *Asian Pac J Trop Bio med.*, 2011; 309-312.
15. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard NCCLS
16. Document M27-A2. Wayne, PA: National Committee for Clinical Laboratory Standards: 2002.
17. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard NCCLS Document M38-A. Wayne, PA: National Committee for Clinical Laboratory Standards: 2002.
18. Singh J, Zaman M, Gupta AK. Evaluation of microdilution and disk diffusion methods for antifungal susceptibility testing of dermatophytes. Med Mycol. 2007; 45: 595-602.

19. Espinel – Ingroff A, etal. Multicenter evaluation of proposed standardized procedure for antifungal susceptibility testing of filamentous fungi. J.Clin. Micol 1997;35:139-143.
20. Sara Carvalhinho, Ana Margarida Costa, Ana Claudia Coelho, Eugenio Martins, Ana Sampaio. Susceptibilities of Candida albicans mouth isolates to anifungal agents essential oils and mouth rinses. Mycopathologia 2012; DOI 10.1007/S 11046-012-9520-4.
21. Esteban A, Abarca ML, Cabanes FJ. Comparison of disk diffusion method and broth microdillution method for antifungal susceptibility testing of dermatophytes. Med Mycol. 2005; 43: 61-6.
22. Fernández-Torres B, Carrillo AJ, Martín E, et al. In vitro activity of ten antifungal drugs against 508 dermatophyte strains. Antimicrob Agents Chemother. 2001; 45(9): 2524-8.
23. Sevtap A. Current status of antifungal susceptibility testing methods. Med Mycol. 2007; 45: 569-87.
24. Santos DA, Barros MES, Hamdan JS. Establishing a method of inoculum preparation for susceptibility testing of Trichophyton rubrum and Trichophyton mentagrophytes. J Clin Microbiol. 2006; 44(1): 98-101.
25. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard NCCLS Document M27-A2. Wayne, PA: National Committee for Clinical Laboratory Standards: 2002.
26. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard NCCLS Document M38-A. Wayne, PA National Committee for Clinical Laboratory Standards: 2002.
27. Singh J, Zaman M, Gupta AK. Evaluation of microdilution and disk diffusion methods for antifungal susceptibility testing of dermatophytes. Med Mycol. 2007; 45: 595-602.
28. Macura AB. In vitro susceptibility of dermatophytes to antifungal drugs: a comparison of two methods. Int J Dermatol. 1993; 32: 533-6.
29. Rubio MC, Gil J, de Ocariz IR, Benito R, Rezusta A. Comparison of results obtained by testing with three different agar media and by the NCCLS M27-A method for in vitro testing of fluconazole against Candida spp. J Clin Microbiol. 2003; 41: 2665-8.
30. Barry AL, Pfaller MA, Rennie RP, Fuchs PC, Brown SD. Precision and accuracy of fluconazole susceptibility testing by broth microdilution, Etest, and disk diffusion methods. Antimicrob Agents Chemother. 2002; 46: 1781-4.

31. Karaca N, Koc AN. In vitro susceptibility testing of dermatophytes: comparison of disk diffusion and reference broth dilution methods. Diagn Microbiol Infect Dis. 2004; 48: 259-64.
32. Keyvan Pakshir, Leila Bahaedinie, Zahra Rezaei, Manuchehr Sodaifi, Kamiar Zomodian. Invitro activity of six antifungal drugs against clinically important dermatophytes . Jundishapur Journal of Microbiology 2009; 2 (4) : 158-163
33. Favre B, Hofbauer B, Hildering KS, Ryder NS. Comparison of in vitro activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a microdilution assay. J Clin Microbiol. 2003; 41: 4817-9.
34. Valerie C, Stanley and Mary P English. Some effects of Nystatin on the growth of four Aspergillus species. J Gen. Microbiol 1965: 40: 107-118.
35. Ball K, Sweeney MP, Baxter WP, Bagg J. Fluconazole sensitivities of candida species isolated from the mouth of terminally ill cancer patients. Am. J.Hosp.Palliat Care 1998: 15(6) : 315-319.