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Original Research Article

Isolation, Identification and Antifungal Susceptibility of Dermatophytes in a Tertiary Care Hospital

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Abstract:

Background and Aim: Dermatophytes have become more common during the last few decades. Although dermatophytosis cannot cause death, it does cause morbidity and is a serious public health concern, particularly in tropical nations like India because to the hot and humid atmosphere. The study's objectives were to isolate and identify dermatophytes from clinically suspected instances of dermatophytosis from skin, hair, and nail samples, as well as to test dermatophyte isolates for antifungal susceptibility.

Material and Methods: The current dermatophytosis investigation was conducted for a year at the Department of Microbiology, Tertiary Care Teaching Institute of India. The study comprised 100 clinically diagnosed cases of dermatophytosis in all age groups and both sexes attending the outpatient department of Dermatology and Venereology. Slide culture, lacto phenol cotton blue mount, hair perforation tests, and urease assays were used to identify the causal bacteria. In this work, we used the CLSI broth microdilution method (M38-A) to assess the efficacy of seven antifungal drugs. Sertaconazole, terbinafine, griseofulvin, fluconazole, itraconazole, voriconazole, and amphotericin B were the antifungals utilised.

Results: Tinea corporis was reported to be the most prevalent clinical manifestation (58%), followed by Tinea cruris (24%). Tinea pedis (7%), followed by Tinea unguium (7%), was the next most common clinical form. Tinea faciei (1%) was the least common kind in our investigation. Out of 100 samples, 61 were KOH positive (61%), while 39 were KOH negative (39%). Out of 100 samples, 32 were culture-positive (32%), whereas 68 were culture-negative (68%).

Conclusion: Dermatophytes are a specialised category of fungus that cause superficial infections in keratinous tissue of humans and other mammals. This study provides an assessment of the prevalence and etiological profile, which may aid in the quantification of the problem and the prevention of dermatophytosis spread by appropriate control methods. Our current research indicates that itraconazole, voriconazole, and griseofulvin are effective against dermatophytes.

Keywords: Dermatophytes, Itraconazole, Tinea corporis, Tinea faci.

Introduction

Dermatophytes are a collection of closely related fungi that can enter the keratinized tissues of skin, hair, and nails and produce an infection known as dermatophytosis, sometimes known as ringworm or tinea. The dermatophytes are included in three fungal genera viz. 1. Epidermophyton, number two. Microsporum, as well as 3. Trichophyton. These fungi colonise keratin tissues and are typically limited to the epidermis's non-living cornified layer. Dermatophytes are also linked to secondary bacterial infections that result in systemic skin diseases. The WHO estimates that the global prevalence of superficial mycotic infection is 20-25%. [1-3] Although dermatophytosis does not cause death, it does cause morbidity and is a major public health problem, particularly in tropical countries like India due to the hot and humid climate. [4] Antimicrobial drug resistance is an unavoidable part of the microbial world's evolutionary process. The earliest resistance in dermatophytes was seen in griseofulvin. [5] Recent studies have shown that some patients are not responding to routinely prescribed drugs such as griseofulvin and terbinafine due to resistance, and

the patients are taking drugs for longer periods of time with no response. [6] Clinically, antifungal drug resistance may be suspected in patients who do not respond to first-line therapy, have a generalized or atypical presentation, and have recurrent episodic. [7,8]

The current work was done to isolate and identify several fungal agents producing mycoses, as detection of these agents is becoming increasingly important for efficient mycosis management. We also used the CLSI broth microdilution method to assess the efficacy of seven antifungal medicines in this investigation. The development of a reference susceptibility testing method may allow the microbiologist to select the proper antifungal medicine and assist the clinician in the treatment of dermatophytic fungus infections.

The study's objectives were to isolate and identify dermatophytes from clinically suspected instances of dermatophytosis from skin, hair, and nail samples, as well as to test dermatophyte isolates for antifungal susceptibility.

Material and Methods

The current dermatophytosis investigation was conducted for a year at the Department of Microbiology, Tertiary Care Teaching Institute of India. The study comprised 100 clinically diagnosed cases of dermatophytosis in all age groups and both sexes attending the outpatient department of Dermatology and Venereology. The institutional ethical committee provided ethical approval, and all participants provided signed informed consent.

Inclusion criteria

Clinical instances with Dermatophytosis of the skin, hair, and nails with no history of topical or systemic antifungal medication in the previous two weeks.

Exclusion criteria

1. Patients with secondary bacterial infections are excluded.
2. Patients undergoing antifungal therapy.
3. Follow-up patients

On a clean glass microscopic slide, a little portion of skin or nail scrapings, nail fragments, or hair roots were inserted. After 10-20 minutes, a drop of 10% KOH was applied and the existence of hyphae and arthrospores was checked. The remaining specimen was grown in sabouraud's dextrose agar containing chloramphenicol and cycloheximide (actidione). Each sample was placed in a separate set of tubes. One tube was incubated at ambient temperature (25-300°C), while the other was incubated at 370°C. Identification was based on colony morphology, rate of growth, pigment production, microscopic appearance, and other relevant tests if growth was obtained on Sabourauds Dextrose agar.

Potassium hydroxide mounts

It is made using the following ingredients: 10gm potassium hydroxide 10ml glycerol 80ml distilled water

Composition of SDA with Cycloheximide and Chloramphenicol

40g Dextrose 10gm Peptone Agar (20gm) Chloramphenicol- 0.05mg/ml in 95% ethanol. Cycloheximide- 0.5 mg/ml in acetone. Distilled water- 1000 ml. The final pH is 5.4. The Lactophenol Cotton Blue (LCB) mount is used to investigate the morphological characteristics of fungal isolates. Simple LCB

It has the following ingredients: 20ml melted phenol 20ml Lactic Acid 40ml glycerol 0.05gm Cotton Blue 20ml distilled water Dermatophytes antifungal susceptibility testing Microdilution of broth6 Microdilution of broth

Requirements

1. Antifungal medications.
2. RPMI-1640 medium with L-glutamine but no sodium bicarbonate.
3. MOPS (3-morpholinopropyl-1-sulfonic acid) buffer.
4. Distilled sterile water.
5. DMSO (dimethyl sulfoxide).
6. Sabourauds dextrose agar.
7. Oat meal agar.
8. Sterile test tubes for drug dilution / inoculum preparation
9. Sterile disposable microtitre plates.
10. Sterile Micropipette / sterile tips / Gloves / disposable face masks.
11. Multichannel pipette.

Medium preparation

Dissolve 10.4 gram RPMI-1640 powder and 34.5 gram MOPS buffer in 900 ml sterile distilled water. Adjust pH to 7.0 using 4M NaOH. Make up to 1 litre with sterile distilled water. Filter sterilizes using 0.22 µ filters. Check sterility and store at 40C.

Concentrations tested

The antifungal drugs are tested over the following range of concentrations-

1. Nine dilutions of each drug were tested;
2. i.e., concentrations of 0.06 to 16 µg/ml for itraconazole, voriconazole, griseofulvin, terbinafine and Sertaconazole
3. Concentrations of 0.25 to 64 µg/ml for amphotericin and fluconazole

Antifungal agent stock preparation

Antifungal stock solutions are prepared at concentration hundred times more than the highest concentration tested.10 ml stock solution was prepared for each drug. Weight (mg) = volume (mL) × desired concentration (mg/mL) Antifungal potency

1. Weigh the appropriate amount of drug required for 10 ml stock.
2. Dissolve the drug in 10 ml DMSO (assuming 100% potency).

Aliquot into 5 × 2 ml snap cap tubes and store at -700Cuntil use. The broth dilution test is performed by using sterile, disposable, multiwall microdilution plates (96 U-shaped wells). To prepare 200 µl of antifungal agent first pipette, 100 µl of RPMI 1640 medium is added to columns 2 to 9. For Fluconazole, 196 µl of RPMI 1640 is added to column 1. Then 4 µl of drug is dispensed into column 1. Serial dilutions are done using multichannel pipette and discard 100 µl from the column 9. Now a column 1 to 9 contains 100 µl solution. Final concentration after inoculation is (128, 64, 32, 16, 8, 4, 2, 1, 0.5)

Inoculum preparation

Dermatophytes are grown on oat meal agar slants until sufficient conidia are present days. These oat meal agar slants are incubated at 300C Overlay agar slant with 3-4 ml sterile distilled water. Gently scrape the surface of the slant with a fungal loop to obtain a conidial suspension. Allow slant to stand for 15-20 min for heavy, hyphal fragments and conidia to settle down. Without disturbing gently transfer the conidial suspension to a fresh tube. Adjust to 0.11 O.D. at 530 nm, and diluted 1:50 in the RPMI media to achieve double concentrated inoculum suspensions of 0.5 × 104 -4.0 × 10 4CFU/ml.

Test procedure

Test was performed in sterile microtitre plates. Aliquots of 100 µl of drug dilutions were already inoculated in columns 1 to 9 microtitre wells. To this add 100 µl of inoculum suspensions to columns 1 to 9. Column 10 is filled with 200 µl of RPMI 1640 medium without drug or inoculum suspension, to serve as sterility control. Column 11 contains 100 µl of the inoculum and 100 µl of drug free RPMI 1640 medium to serve as growth controls.

Incubation

The microtitre plates were incubated at 250C and read after a minimum of 4 days incubation. (5 days for T. rubrum, T. mentagrophytes and T. tonsurans; 7 days for E. floccosum)

Reading results

The MIC was defined (except for Amphotericin) as the point at which there was 80% inhibition of growth as compared with the growth control when read visually in microtitre plates. For Amphotericin, the MIC was measured at 100% growth inhibition compared to growth control. MIC results recorded in µg/ml (CLSI M38-A2).

Statistical analysis

The recorded data was compiled and entered in a spread sheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results

There were 93 skin scrapings and 7 nail clippings among the 100 samples collected. There were no hair samples available. Females had a higher incidence of dermatophytosis. According to this study, the age group of 21-30 years was the most affected by dermatophytosis, followed by 41-50 years. The age group over 70 years old had the lowest incidence.

The majority of male patients were between the ages of 21 and 30, with no cases documented in those aged 70 and up. Females aged 21-30 years and 11-20 years had the highest number of patients, while those aged less than 10 years had the lowest prevalence.

Tinea corporis was reported to be the most prevalent clinical manifestation (58%), followed by Tinea cruris (24%). Tinea pedis (7%), followed by Tinea unguium (7%), was the next most common clinical form. Tinea faciei (1%) was the least common kind in our investigation.

Tinea corporis was more prevalent in males (56.6%) in this study. Tinea cruris (28.78%) and Tinea pedis (7.57%) were found more frequently in females. Males were more likely to have Tinea manuum (5.88%), Tinea unguium (8.82%), and Tinea faciei (2.94%).[Table 1]

Out of 100 samples, 61 were KOH positive (61%), while 39 were KOH negative (39%). (Table 2) Of the 100 samples tested, 32 were culture positive (32%), whereas 68 were culture negative (68%).[Table 3]

Thirty clinically suspected cases of dermatophytosis were confirmed by microscopy and culture. Microscopy revealed 31 instances that were positive but not cultured. Two cases were microscopically negative but culture positive. Thirty-seven cases were found to be negative by microscopy and culture.[Table 4] The sensitivity of

KOH positive to culture was 95%, and the specificity was 55%.

Positive and negative predictive values were 50% and 95%, respectively. Tinea corporis and tinea cruris had the highest number of culture positive. Following that were tinea pedis and tinea manuum.

The isolation rate was 32 (32%), out of 100 patients. The most prevalent species isolated was Trichophyton mentagrophytes (50%) followed by Trichophyton rubrum, Trichophyton tonsurans, Trichophyton verrucosum, and Epidermophyton floccosum. T. mentagrophyes, T. rubrum, T. tonsurans, T. verrucosum, and E. floccosum were tested for antifungal susceptibility. Sertaconazole, Griseofulvin, Terbinafine, Fluconazole, Voriconazole, Itraconazole, and Amphotericin B were among the antifungals examined. Sertaconazole, Griseofulvin, Terbinafine, Fluconazole, Voriconazole, Itraconazole, and Amphotericin B MIC values were all compared to the standard T. mentagrophytes ATCC 4439.

Table 1: Gender Distribution of Clinical Types of Dermatophytosis

Clinical diagnosis	Male		Female		Total
	Number	Percentage (%)	Number	Percentage (%)	
Tinea Corporis	21	61.76	37	56.06	58
Tinea Cruris	5	14.70	19	28.78	24
Tinea Pedis	2	5.88	5	7.57	7
Tinea Manuum	2	5.88	1	1.51	3
Tinea unguium	3	8.82	4	6.06	7
Tinea faciei	1	2.94	0	0	1
Total	34	34	66	66	100

Table 2: Results of KOH study

KOH	Number	Percentage (%)
Positive	61	61
Negative	39	39
Total	100	100

Table 3: Results of culture study

Culture	Number	Percentage (%)
Positive	32	32
Negative	68	68
Total	100	100

Table 4: Relationship between direct smear positivity and culture positivity

KOH	Culture		Total
	Positive	Negative	
Positive	30	31	61
Negative	2	37	39
Total	32	68	100

Discussion

Dermatophytes have evolved into the modern-day sleeping giant. It is one of the oldest fungal infections that has traditionally been overlooked due to the lack of an acute manifestation. The current study examines the clinical pattern and prevalence of various dermatophyte species associated with tinea/ringworm infections in a tertiary care hospital.

According to the current investigation, 32 (32%) of 100 clinically suspected cases of dermatophytosis were isolated by culture. Our findings are close to those of Surekha9, where the isolation rate was 30.8%, and Vikesh Kumar [10], where the isolation rate was 36.6%.Dermatophytosis was more prevalent in participants aged 21-30 in the current study. These findings are consistent with other research in which the most common age group afflicted is 21 to 30 years [11,12,13], and the mean age varies from 30 to 33 years in several studies on dermatophytosis of the skin. [14,15] This could be related to increased physical activity, a higher incidence of injuries, and increased sweating in these individuals, as well as tropical climatic circumstances. Dermatophytic infection was more common in females and less common in males in the current study. This was consistent with the findings of Humera [16] and Sweta et al [17], who found a male to female ratio of 0.85:1 and 0.74:1 respectively. Tinea corporis was found in 58 (58%) of the 100 patients studied. Poojary et al. and other investigations have found a similar pattern, with the most prevalent presentation being a combination of tinea corporis and tinea cruris. [17-19] According to certain studies, tinea corporis is the most prevalent manifestation, while tinea cruris is the least common. [20,21]. In the current investigation,

KOH mount was positive in 61 (61%) of the samples examined and negative in 39 (39%). 32 (32%) of the 100 samples were positive for culture, while 68 (68%) tested negative. This is consistent with the findings of Valarie, Kamothi, and Mahajan, who showed direct microscopic positive of 38.2% and culture positivity of 29.3%, 60% &48.5%, 79.6% &52%, respectively. Hanumanthappa and Lavanya's findings on the link between KOH positivity and cultural positive were equivalent. [20-23]

The distribution of dermatophytosis and its etiological agents vary according to geographical location, community trends, and socioeconomic situations. Trichophyton mentagrophytes was the most common in our sample, followed by Trichophyton rubrum. These findings are congruent with those of Venkatesh V N [24], who found that T. mentagrophyte was the most common dermatophyte (214/13.46%), followed by T. rubrum (55/3.46%).

Antifungal susceptibility testing was performed on 35 Trichophyton spp isolates and 1 Epidermophyton spp isolate against 5 antifungal drugs. The MIC for griseofulvin, fluconazole, voriconazole, itraconazole, terbinafine, amphotericin B, and sertaconazole was determined using the broth micro dilution method, which was modified somewhat from the NCCLS (CLSI) M38A (2007) protocol for antifungal susceptibility testing for filamentous fungi. Terbinafine had a MIC range of 0.06-2g/ml in both T. mentagrophytes and T. rubrum species in this investigation. T. mentagrophytes had higher MICs (2g/ml) in three isolates. This was analogous to Sharma's findings, where the MIC range for sertaconazole was 0.06- 4g/ml. The MIC of griseofulvin was 0.25-1g/ml. Except for a few isolates of T. rubrum, the MIC values of itraconazole and voriconazole were in the 0.06g/ml and 0.06g/ml ranges, respectively. Among all the azoles, fluconazole had shown the highest MIC for all the isolates ranging from 2-8µg/ml. The majority of the isolates had higher MIC ranges for amphotericin B (2-8g/ml).

Conclusion

Dermatophytes are a specialized group of fungi which affect keratinous tissue of humans and of other vertebrates, causing superficial infections. This study provides an assessment of the prevalence and etiological profile, which may aid in the quantification of the problem and the prevention of dermatophytosis spread by appropriate control methods. Our current research indicates that itraconazole, voriconazole, and griseofulvin are effective against dermatophytes. Irregular use of antifungal drugs has led to the emergence of resistant strains, which can cause poor treatment outcomes. Thus it is very important to test for antifungal sensitivity to check for resistance to antifungals.

References

1. Poluri LV, Indugula JP, Kondapaneni SL. Clinicomycological study of dermatophytosis in South India. J Lab Physicians 2015; 7: 84-9.
2. Indira G. In Vitro Antifungal Susceptibility Testing of 5 Antifungal Agents against Dermatophytic Species by CLSI (M38-A) Micro Dilution Method. Clin Microbial, 2014, 3:145.
3. Walke ER, Gaikwad AA, Palekar SS. Clinicomycological profile of dermatophytosis in patients attending dermatology OPD in tertiary care hospital, India. Int J Curr Microbiol App Sci. 2014; 3(10): 432-40.
4. Pai V, Ganavalli A, Kikkeri NN. Antifungal resistance in dermatology. Indian J Dermatol 2018; 63(05):361–368.
5. Hofmann H, Bräutigam M, Weidinger G, Zaun HLAGOS II Study Group. Treatment of toenail onychomycosis. A randomized, double-blind study with terbinafine and griseofulvin. Arch Dermatol 1995; 131(08):919–922.
6. Sahoo AK, Mahajan R. Management of tinea corporis, tinea cruris, and tinea pedis: a comprehensive review. Indian Dermatol Online J 2016; 7(02):77–86.
7. Patel P, Mulla S, Patel D. Gauri Shankar Shrimali. A Study of superficial mycosis in south Gujarat region. National J Comm Med. 2010; 1(2):85–8.
8. Clinical Laboratory Standards Institute / National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Wayne, PA: Approved standard M 38-A. National Committee for Clinical Laboratory; Available from: https:// clsi.org/media/1455/m38a2_ sample.pdf.
9. Surekha G, Kumar KR, Sridevi D, Murty G, Usha G, Bharathi, et al. Superficial dermatomycoses: a prospective clinic mycological study. J Clin Sci Res. 2015; 4(1):7–15.
10. Bhatia VK, Sharma PC. Epidemiological studies on Dermatophytosis in human patients in Himachal Pradesh, India. SpringerPlus. 2014; 3:134. doi:10.1186/2193-1801-3-134.
11. Ansari S, Hedayati MT, Zomorodian K, et al. Molecular characterization and in vitro antifungal susceptibility of 316 clinical isolates of dermatophytes in Iran. Mycopathologia 2016; 181(1-2):89–95.
12. Salehi Z, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Antifungal drug susceptibility profile of clinically important dermatophytes and determination of point mutations in

terbinafine-resistant isolates. Eur J Clin Microbiol Infect Dis 2018;37(10):1841–1846.
13. Poluri LV, Indugula JP, Kondapaneni SL. Clinicomycological study of dermatophytosis in South India. J Lab Physicians 2015;7(02): 84–89.
14. Do NA, Nguyen TD, Nguyen KL, Le TA. Distribution of species of dermatophytes among patients at a dermatology centre of Nghean province, Vietnam, 2015–2016. Mycopathologia 2017; 182(11-12):1061–1067.
15. Arenas R, Moreno-Coutiño G, Vera L, Welsh O. Tinea incognito. Clin Dermatol. 2010; 28(02):137–139.
16. Humera QF, Ansari MB, Patel, Juwairriyyah AW, Siddiqui. Epidemiology and in-vitro antifungal susceptibility testing of dermatophytes in Hyderabad. India. 2014; 2(2):553–560.
17. Prabhu S, Shetty VH, Shetty NJ, Girish P, Rao BK, Oommen RA, et al. Clinico mycological study of superficial fungal infections in coastal Karnataka, India. J Evol Med Dent Sci. 2013; 2(44):8638–46.
18. Poojary S, Miskeen A, Bagadia J, Jaiswal S, Uppuluri P. A study of in vitro antifungal susceptibility patterns of dermatophytic fungi at a tertiary care center in Western India. Indian J Dermatol. 2019; 64 (04):277–284.
19. Verma S, Madhu R. The great Indian epidemic of superficial dermatophytosis: an appraisal. Indian J Dermatol. 2017; 62(03): 227–236.
20. Kamothi MN, Patel BP, Mehta SJ, Kikani KM, Pandya JM. Prevalence of dermatophyte infection in district Rajkot. Electronic J Pharmacol Ther. 2010; 3:1–3.
21. Hanumanthappa H, Sarojini K, Shilpashree P, Muddapur SB. Clinicomycological Study of 150 Cases of Dermatophytosis in a Tertiary Care Hospital in South India. Indian J Dermatol. 2012; 57(4):322–3.
22. Lavanya V, Solabannavar S. Clinico mycological study of dermatophytosis in a tertiary care centre in Bagalkot. Int J Med Health Res. 2015; 1(2):63–6.
23. Mahajan S, Tilak R, Kaushal SK, Mishra RN, Pandey SS. Clinico mycological study of dermatophytic infections and their sensitivity to antifungal drugs in a tertiary care center. Indian J Dermatol Venereol Leprol. 2017; 83(4):436–40.
24. Venkatesh VN, Kotian S. Dermatophytosis: A clinicomycological profile from a tertiary care hospital. J Int Med Dent. 2016; 3(2):96– 102.