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FUNGAL ISOLATES FROM SUPERFICIAL MYCOSES CASES IN ADULTS PATIENTS IN MEERUT

Microbiology

Swati tewari Department of Microbiology, M S Y Medical College & Hospital, Meerut, India

Alok kumar Department of Microbiology, M S Y Medical College & Hospital, Meerut, India

Ekta rani* Department of Microbiology, M S Y Medical College & Hospital, Meerut, India
*Corresponding Author

ABSTRACT

This study was an attempt to estimate the prevalence of fungal isolates in superficial mycoses cases in adults patients in Meerut Uttar Pradesh. A prospective study over a period of one year was conducted from January 2019 to December 2019.The suspected cases of superficial mycoses were subjected to mycological examination with direct microscopy using 10% - 40% KOH depending on the types of samples (skin, nail, hair) processed and culture on Sabouraud's dextrose agar with chloramphenicol and cycloheximide (SDCCA) and also on Potato dextrose agar (PDA) medium. Causative agents were identified macroscopically and microscopically from the growth obtained on SDCCA and PDA. Direct microscopy revealed fungal elements in 252 (91.3%) cases whereas 248 (89.9%) were positive on culture. Tinea corporis 146 (53%) was the most common clinical types and male is to female ratio in relation to clinical types was 2.3:1. Commonest age group affected were 21-30 years with 116 (42.0%) cases. We are reporting change in frequency of dermatophytes isolated from superficial mycoses cases in adult's patients our region.

KEYWORDS

Superficial mycoses, Non- pigmented variants, Dermatophytes

I. INTRODUCTION

Superficial mycoses refer to the disease of the skin and its append ages caused by the fungi. This group includes dermatophytosis, pityriasis versicolor, tinea nigra, white piedra, black piedra and candidiasis1. They are the most common encountered infections in the dermatology outpatient department. Among all the superficial fungal infections, dermatophytic infections have the highest prevalence in the developing countries with significant associated morbidity. Dermatophytic infections are caused by the Trichophyton, Epidermophyton, and Microsporum species. Based on the different body sites involved, the nomenclature varies as tinea corporis, tinea faciei, tinea cruris, tinea pedis, tinea manuum, tinea capitis with Endothrix, and tinea unguium. These infections are predominantly seen in hot tropical countries like India.

Environmental or climatic factors such as the El Nino phenomenon, increased duration of the summer season, increasing humidity, and geographical locations are responsible for the rising incidence of fungal infections. A myriad of host factors contributing to this rising trend are age, race, decreased rate of sebum production, immune status, any disruption in skin barrier, and associated atopic dermatitis2. Low socioeconomic status, poor hygiene, overcrowding, improper sanitation, lack of health education and awareness, and poor health-care facilities are the most important predisposing parameters3. The infections are highly contagious in nature with a high rate of skin-to-skin and fomite transmission and recurrences.

II. MATERIALS AND METHODOLOGY

This is a one year (January 2019 to December 2019) prospective study, was conducted at Mulayam singh yadav medical college and hospital, Meerut. The study population comprised of 276 clinically suspected cases of superficial mycoses attending Dermatology outpatients department at Mulayam singh yadav medical college and hospital, Meerut. All the clinically suspected cases of superficial mycoses referred to Department of Microbiology for isolation and identification of etiological agent were included in the study. Demographic details of every case and detailed history of onset of disease, duration of symptoms, trauma, patient's occupation, drugs, associated co-morbid conditions, family and personal history was taken. Enquiries were also made as to exposure to animals, cases or any other suspected sources.

III. SPECIMEN COLLECTION AND PROCEDURE

The affected areas were swabbed with 70% alcohol. Skin scrapings or nail clippings or plucked hair was collected in clean white paper packets.

Skin scraping:

Skin scrapings were collected by scraping across the inflammatory margin of the lesion including the healthy skin using sterile scalpel or clean slide. If vesicles are present, the top was removed with fine scissors and stored for further examination.

Nail scraping:

Nail specimen was collected by taking the infected nail clippings and was scraped deeply enough to obtain the recently invaded nail tissue. In cases of paronychia i.e. where a yeast infection is suspected, exudate was expressed from the Paronychial folds by probing with a flat excavator and collecting on a swab previously moistened with sterile saline.

Hair plucking:

Hair specimen was collected by plucking the infected hair including the base of hair shaft. The species most frequently associated with scalp ringworm cause the affected hairs to fluoresce under a wood's lamp and this is a useful means of selecting material.

Direct microscopic examination

KOH mount: 10% KOH solution was used for skin and hair samples.40% KOH for nail specimens and incubated overnight at 37ºc for clearing. Clearing can be hastened by gently heating. As soon as the specimen has cleared, examined under microscope using the 10x and 40x objectives, for the presence of filamentous, septate, branching hyphae with or without arthrospores. In case of hair, type and arrangement of spores were noted (ectothrix/ endothrix). Tinea versicolor infections were diagnosed by the presence of round yeast cells with short, stout and curved hyphae (spaghetti and meat ball appearance).

Fungal culture

All the samples were collected and inoculated on two sets of test tubes containing Sabouraud's dextrose agar with chloramphenicol and cycloheximide and Potato dextrose agar. For Tinea versicolor infections SDA with sterile olive oil overlay was used. The fungal cultures have been identified by colony morphology, rate of growth and pigment production. Lactophenol cotton blue mount was done from the small bit of colony taken on clean glass slide and teased out using two teasing needles, to detect the presence of macroconidia, microconidia, chlamydospore and special hyphal structures. Confirmatory identification of the species was done by slide culture technique and Biochemical tests.

IV. OBSERVATIONS AND RESULTS

A total of 96 patients were enrolled in the study, comprising 186 males (67.3%) and 90 females (32.6%). None of them had any systemic diseases. Tinea corporis 146 (53%) was the most common clinical type seen (table 1) and the male to female ratio in relation to clinical types was found to be 2:1 which was significant (p=0.0001) (table 2).

Table 1: Distribution of clinical types of superficial mycoses cases

Clinical types	Total N (%)
Tinea corporis	146 (53)
Tinea cruris	45 (16.3)
Tinea capitis	28 (10.1)
Tinea pedis	21 (7.6)
Tinea unguium	15 (5.4)
Tinea faciei	11 (4.1)
Tinea manuum	10 (3.6)
TOTAL	276(100)

Table 2: Gender distribution of clinical types

Clinical types	No. of cases N (%)	Male N (%)	Female N (%)	M:F ratio
T. corporis	146(53)	102	44	2.3:1
T. cruris	45(16.3)	29	16	1.8:1
T. capitis	28(10.1)	21	07	3:1
T. pedis	21(7.6)	13	08	1.6:1
T. unguium	15(5.4)	09	06	1.5:1
T. manuum	11(4.1)	06	05	1.2:1
T. faciei	10(3.6)	06	04	1.5:1
TOTAL	276(100)	186(67.3)	90(32.6)	2:1

Out of total 276 clinical samples processed, found 252 (91.3%) were positive by direct microscopy (KOH mount) and 248 (89.8%) were culture positive. Whereas, 04 (1.4%) samples were found to be KOH positive but culture negative, while zero(00) were KOH negative samples were culture positive. 24 (8.7%) samples were negative by both KOH and culture (table 3). Culture negative samples were excluded while calculating the prevalence of fungal isolates.

Table 3: Comparative results of KOH & culture

KOH mount	Total	Culture	
	N (%)	Positive N (%)	Negative N (%)
Positive	252(91.3)	248(89.8)	04(1.4)
Negative	24(8.7)	00	24(8.7)
Total	276(100)	248(89.8)	28(10.1)

On direct microscopic examination by KOH mount, out of the total 276 samples, 252 (91.3%) samples were positive, while only 24 (8.7%) were negative, and 248 (89.9%) were culture positive and 04 (1.4%) were negative. All 24 KOH negative samples turned out to be culture negative too.

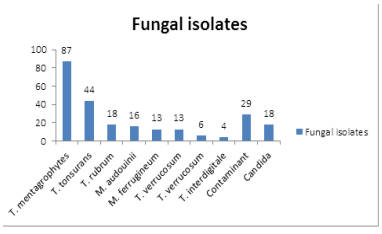

Figure 1: Bar diagram showing frequency of fungal isolates from culture positive samples

Table 4: Gender distribution of fungal isolates

Fungal isolates	Total N (%)	Male N (%)	Female N (%)
T. mentagrophytes	87(35.1)	49(19.8)	38(15.3)
T. tonsurans	44(17.7)	26(10.5)	18(7.3)
T. rubrum	18(7.3)	13(5.2)	5(2.0)
M. audouinii	16(6.5)	11(4.4)	5(2.0)
M. ferrugineum	13(5.2)	11(4.4)	2(0.8)
T. schoenleinii	13(5.2)	10(4.0)	3(1.2)
T. verrucosum	06(2.4)	5(2.0)	1(0.4)
T. interdigitale	4(1.6)	4(1.6)	00
Contaminant	29(11.7)	16(6.5)	13(5.2)
Candida	18(7.3)	14(5.6)	4(1.6)
TOTAL	248(100)	159(64.1)	89(35.9)

On analysing gender distribution of fungal isolates, a difference of double was found as majority 54 (64.1%) were from males and only 89 (35.9%) from female cases.

Eighty seven isolates of T. mentagrophytes were approximately equally distributed among 49 (19.8%) males and 38 (15.3%) females. T. tonsurans isolates predominated in males with double frequency of 26 (10.5%) compared to only 18 (7.3%) isolates from females. 18 (7.3%) isolates of T. rubrum followed similar pattern as that of T. tonsurans and was predominantly isolated from 13 (5.2%) males compared to 5 (2.0%) female. T. schoen-leinii isolates also exhibited the pattern of above mentioned species and predominated in males 10 (4.0%) compared to females 3 (1.2%).

V. DISCUSSION

Out of total 276 cases of superficial mycoses, 252 (91.3%) were positive in direct microscopic examination (KOH). 248 (89.6%) were culture positive. Similar findings were reported in other studies also, which is mentioned in (Table 5).

Table 5: Comparative analysis of KOH

KOH Results	Present study	Madhulika et al.,9 2014 (West Bengal)	Vyas et al.,10 2013 (North India)
Positive	91.3%	75.7%	62.5%
Negative	8.7%	24.3%	37.5%

We found an isolation rate of 248 (89.6%) among the samples which showed positive KOH mount. Out of 248 (89.6%) culture positive samples no were negative on microscopy (KOH mount). Similar findings were reported in other studies which are mentioned in (Table 6).

Table 6: Comparative analysis of culture result from previous studies

Culture	Present study	Madhulika et al.,9	Vyas et al.,10 2013	Singh and Beena.,17 2003 (Baroda)
Results		2014	(North India)	
Positive	89.6%	46.28%	37.5%	44.62%

In the present study, Trichophyton mentagrophytes was the predominate isolate followed by T. tonsurans, T. rubrum, M. audouinii, M. ferrugineum and T. schoenleinii with the frequency of 35.1%, 17.7%, 7.3%, 6.5% and 5.2% respectively. However, we did not observe any involvement of Epidermophyton species in the study.

Majority of the studies have reported T. rubrum as main dermatophytic isolate from superficial mycoses cases in India. However, we have found T. mentagrophytes as predominant species followed by T. tonsurans and T. rubrum. Our findings are similar to recent studies done by others. and hints towards change in frequency of dermatophytes in our region.

Table 6.8: Comparative analysis of commonest fungal isolates

Clinical isolate	Present study	Agarwal et al.,25 2014 (North-west India)	Bhatia & Sharma.,19 2014 (Himachal Pradesh)	Sahai & Mishra.,26 2011 (Central India)
T. mentagrophytes	30.9%	37.9%	63.5%	25%
T. tonsurans	21%	8.3%	-	20%

VI. CONCLUSION

Our study has given us insights into the clinic-mycological aspects of superficial mycoses in our region. The study reveals that skin infections are more common than the hair and nail infections in dermatophytoses cases. Common clinical types are T. corporis, T. pedis, T. manuum and T. cruris. Unhygienic conditions among low-socioeconomic group, frequent migration of labourers, workers to this region may be some of the contributing epidemiological factors. Dermatomycoses was seen in 93.5% and superficial candidiasis in 6.5% cases. T. mentagrophytes was implicated as the predominating species followed by T. tonsurans, T. rubrum, M. audouinii, T.

schoenleinii, Aspergillus terreus, Alternaria species, Fusarium species, Candida albicans, Candida non-albican, Scytalidium species and Mucor species. Isolation of non-pigmented variants of T. rubrum confirms our findings of change in distribution of dermatophytes in our region. India is a growing economy and during last couple of decades interstate migration of population and increase in national and foreign tourism might be an important reason for change in frequency of dermatophytes and uncommon fungal isolates in clinical practice. We are reporting a change in frequency of dermatophytosis isolated from superficial mycoses cases in our region. However, the present study is a small study that focuses primarily on the prevalence of different dermatophytes species in Northern Meerut and a systematic study covering larger population and over a longer period of time would give a better insight into the epidemiology of dermatophytes in Meerut and neighbouring region.

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