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Isolation of Dermatophytes from Clinically Suspected Cases of Superficial Fungal Infections

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A B S T R A C T

Dermatophytes are keratinophilic fungi responsible for dermatophytoses which are superficial mycoses affecting skin, hair and nails. The aim of the study was to isolate dermatophytes from clinically suspected cases of superficial fungal infections. This study was carried out at the Department of Microbiology, Grant Government Medical College & Sir J.J Group of Hospitals, Mumbai. A total of 279 clinically suspected cases of superficial fungal infections were included in the present study. The specimens like skin scraping, nail and hair were collected with all aseptic precautions and were first examined under microscope on KOH mount and then inoculated on to duplicate slopes of Sabouraud dextrose agar (plain) and SDA (Chloramphenicol and Cycloheximide). Lactophenol cotton blue preparations were made of each fungal growth and were identified using specific tests like slide culture, hair perforation and urease test. Out of the 279 clinically suspected cases of superficial mycoses, maximum involved the skin (65.23%) and a total of 216 (77.42%) cases were of clinically suspected dermatophytoses. KOH mount examination alone could detect 144 (66.66%) cases out of the 216 clinically suspected cases of dermatophytoses. A total 162 isolates were confirmed in which maximum dermatophytes 122 (75.30%) were found. Amongst the dermatophytes, *T. rubrum* was the commonest pathogenic species isolated followed by *T. mentagrophytes*. Other species isolated were *T. tonsurans*, *M. gypseum*, *E. floccosum*, *T. verrucosum* and *M. audouinii*, *T. rubrum* and *T. mentagrophytes* were mainly isolated from *Tinea unguium* followed by *Tinea corporis* cases. *M. gypseum* was isolated only from Tinea capitis cases whereas *M. audouinii* was isolated only from *Tinea unguium* cases. Dermatophytoses are the most common types of superficial cutaneous fungal infections. The incidence of Dermatophytoses is increasing in India due to widespread and indiscriminate use of corticosteroids and antifungal agents without performing appropriate microbiological investigations.

Introduction

The present study was undertaken for the isolation of dermatophytes from clinically suspected cases of superficial fungal infections. Human fungal infections can be divided into two broad categories: Superficial fungal infections and Deep seated (systemic) fungal infections. Superficial mycosis can be defined as fungal infection of the superficial part of the skin. [1]Superficial infections are by far commoner and comprise of the various types of tineas or ringworms. Superficial infections are of two types: a. Surface infections comprising of *Pityriasis versicolor, Tinea nigra* and *Piedra. b. Cutaneous* infections.

Most important cutaneous infection is dermatophytosis caused by a group of related fungi called the dermatophytes. Dermatophytes are the members of the ascomycetes subgroup which being both keratolytic and keratophytic, cause a wide range of dermatological manifestation, often called ringworm or tineas. [2]These are the infections of keratinized tissues caused by three groups of dermatophytes, classified as Anthropophilic, Zoophilic and Geophilic according to their preference to man, animal and soil.

All may cause infections in humans. Within these groups, the three genera *Trichophyton*, *Microsporum* and *Epidermophyton* are recognized. [2]The common sites of infection are the keratinized tissues such as the stratum corneum of the epidermis, nails and hair. Dermatophytosis in India has received increasing attention in recent years from different parts of the country. The Indian subcontinent has a remarkable varied topography and as a monsoon land, most parts of the country experience sustained periods of a combination of recurrent heat and high humidity. Such conditions favour the occurrence of mycotic infections. The fungi causing dermatophytoses vary from place to place and seasonal variations also have been observed.[2]

Materials and Methods

This was a prospective study carried out at the Department of Microbiology, Grant Government Medical College & Sir J.J Group of Hospitals, Mumbai over a period of 18 months.

A total of 279 clinically suspected cases of superficial fungal infections attending Outpatient department and Indoor patient department were included in the present study.

Specimen collection

The specimens like skin scraping, nail and hair were collected in sterile paper packets with all aseptic precautions.

Skin scrapings

After cleaning the lesion with 70% alcohol, scrapings were collected aseptically from the active border of the lesions using sterile scalpel in a sterile paper packet. Care was taken to avoid bleeding.

Nail

Recently invaded, deformed, brittle pigmented nails were selected and site was cleaned with 70% alcohol. By using nail clippers, infected portions of the nails were collected in sterile paper packets.

Hair

Site of lesion was cleaned with 70% alcohol. Infected, brittle, lustreless hair stumps were removed with forceps and collected in sterile paper packets.

Laboratory examination

Direct demonstration of fungal elements

A small portion of specimen was mixed with a drop of 10% KOH and kept for 10 to 15 minutes. In case of nails, 40% KOH was used and kept for 24 hours. Preparation was warmed and observed under microscope using low and high magnification for the demonstration of branching filaments and spores.

Culture

The specimens collected were inoculated on to duplicate slopes of Sabouraud dextrose agar(plain)(pH 5.6±02) and SDA slopes containing Chloramphenicol and Cycloheximide using sterile precautions irrespective of demonstration of fungal elements on KOH mount. One set of above slopes were incubated at 37°C and other set of slopes at room temperature. All the culture slopes were examined regularly for a period of four weeks for the presence of fungal growth in relation to their size, surface, texture, pigmentation on both sides (obverse and reverse) colour and margin of the colony. Findings were recorded weekly for a period of four weeks and slope without growth were discarded after 28 days. After incubation of slopes all colony characters were noted. Lactophenol cotton blue preparations were made of each fungal growth and were identified using specific tests like slide culture, hair perforation and urease test.

Slide culture technique

A slide was placed on a bent glass rod in the petri dish and sterilised. SDA plates were prepared and with the help of sterile scalpel, agar blocks were cut and placed on a slide in petri dish with sterile precautions. The fungal growth was inoculated towards the centre from the four sides of agar block. The blocks were covered with sterile cover slips. About 6-7 ml sterile distilled water was added to the bottom of petri dish to maintain the moisture and incubated at 25°C until growth occurred. When growth appeared, the cover slip was removed and placed aside with fungus growth upwards. A drop of 95% ethyl alcohol was placed to fix the fungus on slide and cover slip, it was allowed to dry. A drop of lactophenol cotton blue was placed on the slide and covered with clean cover slip. On another slide a drop of lactophenol cotton blue was placed and covered with original cover slip with mycelial surface down. Excess mounting fluid was blotted away from cover slip of the two preparations. Edges were sealed with nail polish and observed under low and high magnification of microscope for microconidia as well as macroconidia.

Urease test

Modified Christensen's urea agar were inoculated with the fungal growth and incubated at 37°C for 7-14 days to differentiate *T. rubrum* and *T. mentagrophytes* species. *T. mentagrophytes* hydrolyse urea thereby medium turns deep red giving positive results while *T. rubrum*does not hydrolyse urea giving negative results.

Hair perforation test

T. mentagrophytes and *T. rubrum* were differentiated as well as *M.canis* and *M. equinum*, respectively by implanting a few healthy hair stumps with fragments of fungal colony in sterile petri dish with 25 ml of sterile distilled water and 5-6 drops of 1% yeast extract.

Incubation was done at 28°C and hair stumps were observed periodically for the presence of wedge-shaped perforation under the microscope for a period of 4 weeks which is

positive in cases of *T. Mentagrophytes* and *M. canis* while *T.rubrum* and *M.equinum* show negative results.

Results and Discussion

Out of the total 279 cases of clinically suspected superficial fungal infections, 182 (65.23%) involved the skin, 86 (30.82%) involved the nail while 11 (3.94%) involved the hair.

A total of 216 (77.42%) cases were of clinically suspected dermatophytoses out of the total cases of clinically suspected superficial mycoses while Tinea versicolour and Candidiasis accounted for 35 (12.55%) and 28 (10.03%) cases respectively.

KOH mount examination alone could detect 144 (66.66%) cases out of the 216 clinically suspected cases of dermatophytoses. Also, out of the total 216 clinically suspected cases of dermatophytoses, 114 (52.77%) were diagnosed by both, KOH examination and culture. 30 (13.88%) cases of dermatophytosis were confirmed by KOH mount examination, however failed to grow on culture. 17 (7.87%) KOH negative cases of clinically suspected cases of dermatophytosis showed growth on culture while 55 (25.46%) cases of dermatophytes were both KOH and culture negative.

A total 162 isolates were confirmed from the 279 clinically suspected superficial mycoses cases, in which maximum dermatophytes 122 (75.30%) were found. This was followed by 18 *Malassezia* species (11.11%), 13 *Candida* species (8.02%), 7 *Aspergillus* species (4.32%) and 2 *Mucor* species (1.23%) respectively. Out of the total 122 isolated dermatophytes, 110 (90.16%) *Trichophyton* species, 4 (3.27%) *Epidermophyton* species and 8 (6.55%) *Microsporum* species were isolated. *T. rubrum* was the commonest pathogenic species isolated (n=56/45.90%) followed by *T.mentagrophytes* (n=45/36.89%). *T. tonsurans* and *M.gypseum* isolates were found equally (n=6/4.92%). There were (n=4/3.28%) isolates of *E. floccosum*, (n=3/2.46%) isolates of *T. verrucosum* and (n=2/1.64%) isolates of *M. audouinii* respectively. (Table 1)

Table.1 Distribution of dermatophyte species

Dermatophyte species	Number	Percentage
***Trichophyton* species (n=110) (90.16%)**		
T. rubrum	56	45.90
T. mentagrophytes	45	36.89
T. tonsurans	6	4.92
T. verrucosum	3	2.46
***Epidermophyton* species (n=4) (3.27%)**		
E. floccosum	4	3.28
***Microsporum* species (n=8) (6.55%)**		
M. gypseum	6	4.92
M. audouinii	2	1.64
TOTAL	**122**	**100**

Table.2 Disease wise distribution of isolated strains of dermatophytes

Clinical pattern	Total samples	*T. rubrum*	*T. mentagrophytes*	*T. tonsurans*	*T. verrucosum*	*E. floccosum*	*M. gypseum*	*M. audouinii*	Total isolates
T. cruris	**23**	6	5	0	0	0	0	0	**11**
T. corporis	**44**	13	12	4	0	0	0	0	**29**
T. cruris + corporis	**26**	6	6	1	0	0	0	0	**13**
T. unguium	**86**	23	19	0	2	3	0	2	**49**
T. capitis	**15**	2	1	0	1	0	6	0	**10**
T. pedis	**12**	5	1	0	0	0	0	0	**6**
T. manuum	**6**	1	0	0	0	1	0	0	**2**
T. faciei/ T. barbae	**4**	0	1	1	0	0	0	0	**2**
Total	**216**	**56**	**45**	**6**	**3**	**4**	**6**	**2**	**122**

T. rubrum and *T. mentagrophytes* were mainly isolated from *Tinea unguium* followed by *Tinea corporis* cases. *T. tonsurans* was maximally isolated from *Tinea corporis* whereas *E.floccosum* and *T. verrucosum* were maximally isolated from *Tinea unguium* cases. *M.gypseum* was isolated only from *Tinea capitis* cases whereas *M. audouinii* was isolated only from *Tinea unguium* cases (Table 2).

This study was carried out at the Department of Microbiology, Grant Govt. Medical College & Sir J.J Group of Hospitals, Mumbai from January 2016 to June 2017 for the isolation of dermatophytes from clinically suspected cases of superficial fungal infections.

In the present study, a total of 279 clinically suspected cases of superficial mycoses were recorded. Samples from all 279 cases were subjected to culture on Sabouraud Dextrose agar and followed up for growth.

Out of the 279 cases, 182 (65.23%) of the recorded fungal infections involved the skin, 86 (30.82%) involved the nail while 11 (3.94%) involved the hair. In the study conducted by Abida Malik *et al.,* in 2014, commonest clinical presentation in the study was skin infection 257 (60.4%) followed by nail infection 161 (37.8%) and hair infection 7 (1.61%) which correlated well with our study.[3]

In the present study, based on different types of lesions on skin, nail and hair, it was observed that dermatophytoses constituted 77.42% of superficial mycoses. Among the nondermatophytic infections, tinea versicolor (12%) and candidiasis (10.03%) were recorded. In the study conducted by Wg Cdr Sanjiv Grover *et al.,* dermatophytosis was the commonest superficial fungal infection in 103 cases (70.5%), followed by candidiasis in 30 (20.5%) and Pityriasis versicolor in 13 (9.0%) cases which also correlated well with our study.[4]

Of the total of 216 clinically suspected cases of dermatophytic infections, 144 (66.66%) were detected on KOH mount examination alone. Overall, only 52.77% cases of dermatophytoses, 51.42% cases of tinea versicolor and 42.85% cases of candidiasis could be diagnosed by both KOH mount examination and culture combined. In all three clinical types, there were many cases which could be diagnosed either by KOH mount examination or by culture alone. Therefore, it was observed that it is important to carry out both KOH mount examination and culture in all types of cases of superficial mycoses.

In the present study, 75.31% of the total isolates were dermatophytes (122/162), followed by 11.11 % of *Malassezia* species (18/162) and 8.02% of *Candida* species (13/162) which correlates with the study conducted by P Kannan *et al.,* in which eighty out of 165 cases (48.5%) were dermatophytoses, 39 (23.6%) were pityriasis versicolor, 29 (17.1%) were candidiasis and 12 (7.1%) were cases of mycetoma.[5]

Dermatophytes are the major cause of cutaneous mycoses and remain a general public health problem. In the present study, dermatophytes were the predominant isolates recovered from the clinically suspected cases of superficial fungal infections (75.31%). In the present study, *Trichophyton* species were the most common dermatophytes isolated (90.16%) followed by *Microsporum* species (6.55%) and *Epidermophyton* species (3.27%). Amongst the *Trichophyton* species, *T. rubrum* was the commonest (45.90%), followed by *T.mentagrophytes* (36.89%). Other species included *T. tonsurans* and *M. gypseum* each (4.92%), *E. floccosum* (3.28%), *T. verrucosum* (2.46%) and *M. audouinii* (1.64%) (Table 1). Other workers who reported *T. rubrum* as predominant isolate in their studies, were Singh S *et al.,* in 2003 – 73.27%, [6] Mohanthy JC *et al.,* in1998 – 68.34%, [7] Bindu *et al.,* in

2002-66.2%, [8] Sumana *et al.*, in 2004 – 60%, [9] Peerapur *et al.*, in 2004 – 43.7%, [10] Gupta BK *et al.*, in 1993 -42.42%.[11]

In the present study the commonest clinical dermatophytic manifestation was *Tinea unguium* (40.16%) followed by *Tinea corporis* (23.77%). However, taking into account cases of *Tinea cruris* and cases of mixed infections (*Tinea cruris* and *Tinea corporis*), these two clinical types constituted 43.05% of the total cases of dermatophytoses. (Table 2) The isolation rate of various dermatophytes from the 93 cases of *Tinea cruris* and *Tinea corporis* was 56.98%. Similarly, the isolation rate of various dermatophytes in the 86 cases of *Tinea unguium* was 56.97%. The studies conducted by Venkatesan *et al.*, in 2007 (64.8%), [12]Nita Patwardhan *et al.*, in 1999 (24.5%), [13]Sumana and Rajagopal in 2002 (48.66%) [14] show *Tinea corporis* as the most common dermatophytic manifestation.

The use of tight undergarments, high rate of sweating in groin and waist regions and constant sweating coupled with lack of personal hygiene makes these sites more vulnerable to dermatophytoses.

Superficial fungal infections are very common in India due to several contributing factors like hot and humid climate, poor hygienic conditions, over population and immunosuppression. The incidence of Dermatophytoses is increasing in our country due to widespread and indiscriminate use of corticosteroids and antifungal agents without performing appropriate microbiological investigations. All these factors, play a significant role in the growth of these fungi.

Dermatophytes were the predominant isolates (75.31%) recovered from the clinically suspected cases of superficial fungal infections. Early diagnosis and treatment can limit the spread of these infections. Therefore, a proper clinical diagnosis, supported with a robust laboratory with expertise in performing KOH mount examination and carrying out culture in all cases of superficial mycoses is important. This will help in guiding the clinician in selecting appropriate antifungal agents for treatment. Also, many cases of superficial mycoses remain undetected on KOH mount examination or fail to grow on culture, therefore following the proper method of sample collection is of utmost importance for diagnosis.

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