Original Research Article

CLINICOMYCOLOGICAL PROFILE OF DERMATOPHYTES AND THEIR PREVALENCE AT TERTIARY CARE HOSPITAL, CHHATRAPATI SAMBHAJINAGAR, MAHARASHTRA, INDIA

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ABSTRACT

Background: Dermatophytosis is the commonest contagious fungal infection seen in man & animals which affects skin, hair & nail. The patients usually neglect such infections and seek medical attention for cosmetic purposes. The clinical presentation is typical of Ringworm infection (taenia), but can be confused with other skin disorders due to rampant application of steroids containing skin ointments and creams leading to misdiagnosis and mismanagement. **Aims & Objectives:** To assess the clinicomycological profile of dermatophytoses and identify the species of dermatophytes. To study risk factors responsible for dermatophytosis. To co-relate the clinical pattern of dermatophytosis with the isolated dermatophytes.

Materials and Methods: Samples of skin, hair & nail from clinically suspected cases of dermatophytosis were taken as per standard guidelines. The identification of causative fungus was done from the colony characteristics on SDCCA (Sabouraud's dextrose agar containing Chloramphenicol & Cyclohexamide), rate of growth, microscopic features and results of various specific tests.

Results and Conclusion: In this study clinically suspected 138 cases of dermatophytoses were included. Samples of skin were 86 (62.31%), nails were 45 (32.60%), and hairs were 7 (5.07%). Out of these, 73 (52.89%) were found to be KOH (Potassium Hydroxide) with DMSO (Dimethyl Sulphoxide) positive and 65 (47.10%) were KOH with DMSO negative; 61 (44.20%) were culture positive & 77 (55.79%) were culture negative. Amongst culture positive samples, dermatophytes were isolated in 46 (75.40%) cases, non dermatophyte molds in 11 (18.03%) cases & candida in 4 (6.55%) cases. In the present study, *Trichophyton mentagrophytes* (50%) was the predominant isolate followed by *Trichophyton rubrum* (43.47%), *Microsporum gypseum* (4.34%) & *Trichophyton tonsurans* (2.1%) in the dermatophytes.

Keywords: Dermatophytosis, Dermatophyte Test Medium, Taenia, Ringworm infection.

INTRODUCTION

Dermatophytosis is the commonest contagious fungal infection seen in man & animals which affects skin, hair & nail. Most common clinical presentation of dermatophytosis is circular reddish rash with itching and scaling with central clear area.[1,2,3] They are commonly known as ringworm infections or Taenia.[1,4,5] These problems are not generally life threatening but they are the most common diseases of mankind. These superficial skin infections are attributed to two types of fungi, dermatophytes, and Malassezia.[6] Dermatophytic fungal species belong to three genera:[2,4,7]

1. Trichophyton species infect skin, hair & nail.
2. Microsporum species infect skin & hair only.
3. Epidermophyton species infect skin & nail only.

These fungi produce proteases (keratinase) enzyme that digest keratin,[8] & allow colonization & infection of stratum corneum of skin, hair & nail,[9,10] but do not penetrate in deeper anatomical sites.[11] They can invade the hair follicles, causing folliculitis or perifollicular abscesses (kerion celsi).[8] The patients usually neglect such infections and seek medical attention for cosmetic purposes.[11] When host's immunity is hampered the fungus may invade deeper layers of skin and multiply to develop inflammatory granuloma called as tinea profunda.[8]

Dermatophytes are more prevalent in tropical & subtropical countries including India where heat & moisture play an important role in promoting growth of Dermatophytic fungi.[2,7,12,13] Dermatophytosis constitutes about 10 % of all skin diseases.[10] Overcrowding, lack of personal hygiene and exposure to animals or cases are some of the risk factors which promote the dermatophytic infections.[2,8] It also include people who use communicable bath and who are involved in contact sports such as wrestling.[3] Decreased cellular immunity due to malignancy, administration of immunosuppressive drugs, endocrine disorders like Cushing's can lead to invasive dermatophytic infections.[8,14] In patients with organ transplantation, leukemia and HIV infectivity,[15] there is increase in incidence of fungal infections due to frequent usages of higher antibiotics. Fungal infections have attracted the attention of Physicians and Microbiologists in recent years due to various reasons like indiscriminate use of antibiotics, anticancer therapy and immunodeficient diseases like AIDS.[12,13]

The clinical presentation is very typical of Ringworm infection, mostly diagnosis is made on clinical grounds, but confused with other skin disorders due to rampant application of steroids containing skin ointments and creams leading to misdiagnosis and mismanagement.[7,16] Most of the infections are not life threatening but they cause morbidity in immunocompromised and diabetic patients.[3,17] Grossly the lesions include outer ring of active progression with central healing with itching, redness, scaling or fissuring of the skin. Aggressive infections may lead to an abscess or cellulitis formation.[7] Any clinical diagnosis needs to be supported by laboratory diagnosis. For the identification of etiological agent culture is necessary along with direct microscopic examination.[14]

In view of above, identification of organism is essential with its prevalence in particular geographical area and association of clinical presentation with isolate to treat such patients with appropriate antifungal agent. Knowing the risk factors will help us in defining preventive measures to avoid dermatophytic infections.

Aims and Objectives

To assess the clinicomycological profile of dermatophytoses and identify the species of dermatophytes. To study risk factors responsible for dermatophytosis. To co-relate the clinical pattern of dermatophytosis with the isolated dermatophytes.

Inclusion Criteria

Clinically suspected cases of dermatophytoses (skin, hair & nail infections) of all age groups & of both sexes.[3]

Exclusion Criteria

Fungal diseases other than dermatophytoses.

Cases of dermatophytoses with secondary bacterial infections.

Sample Size Calculation: Sample size calculated by using below formula.

$$n = \frac{z^2 p(1-p)}{d^2}$$

n = sample size

p =prevalence or incidence = 10% = 0.10

d = allowable error =5% = 0.05

z = 1.96 for 95% C.I.

Study Populations: 138 specimens from clinically suspected cases of dermatophytosis were studied.

MATERIALS AND METHODS

The present study was carried out in the department of Microbiology with cooperation of dermatology outpatient department after getting Ethics Committee approval. Total 138 specimens from clinically suspected cases of dermatophytosis were studied. History of these patients was taken with reference to age, occupation, hobbies, living conditions etc as per case record form.

Samples of skin, hair & nail from clinically suspected cases of dermatophytosis were taken as per standard guidelines.[1]

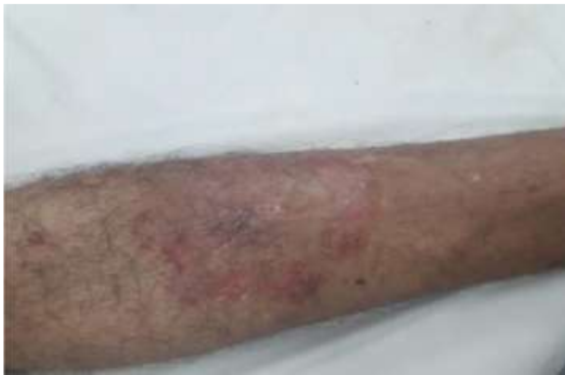

Figure a: annular lesion on forearm

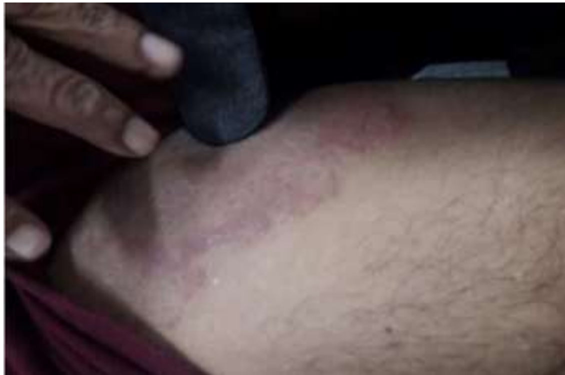

Figure b: annular lesion on thigh

Direct microscopic examination was performed using 20% KOH with DMSO for the detection of fungal element like hyphae, pseudohyphae, yeast cells, spores, and spherule or sclerotic bodies. KOH dissolves the keratinized material of cells, digests proteinaceous debris and thus clears up the background against which fungi are clearly seen and DMSO acts as useful cleansing agent and helps fasten the clearing of material.[1,18] So the preparation can be examined immediately and heating of the preparation was not required.

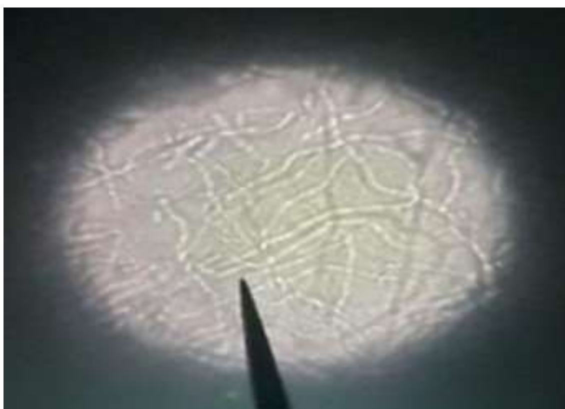

Figure c: KOH mount examination showing hyphal elements

Fungal culture: Fungal culture was done by inoculating specimen on two slopes of SDCCA and incubated at two temperatures 25^0C & 37^0C to facilitate growth. The rate of fungal growth was assessed. The culture slopes showing fungal growth were examined for colony characters, appearance, and surface, colour and texture of colony. The pigment production was observed on reverse side of culture slopes and was recorded. Growth was examined for fungal growth daily up to one week and biweekly up to three weeks.[1] The slopes showing no growth were discarded after 21 days and declared as negative for culture.[1] Simultaneously a part of specimen was inoculated on DTM (Dermatophyte Test Medium) and was kept at 25^0C. The DTM media after inoculation was examined for change in colour from yellow to red within 3 to 6 days.[1,3]

Figure d: Growth of Trichophyton rubrum on SDCCA(Tube)

Figure e: Growth of Trichophyton mentagrophyte on SDCCA (Plate)

Figure f: growth of Trichophyton rubrum on DTM

The LPCB (Lacto Phenol Cotton Blue) wet mount preparation was done from colony to observe the morphological details microscopically. Findings were noted in like shape, size of microconidia, macroconidia and their relation to the hyphae, presence of arthrospores, presence of different types of hyphae like spiral, pectinate and antler hyphae.[1]

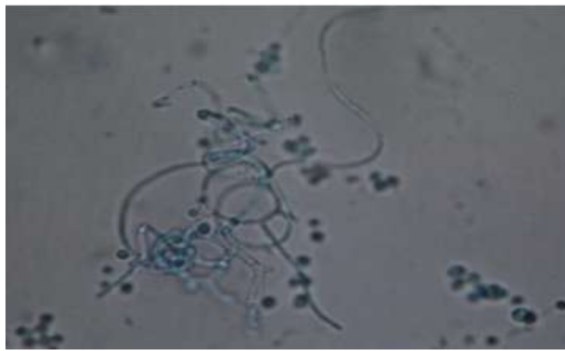

Figure G: LPCB mount examination showing hyphal elements (spiral hyphae)

Slide cultures, Hair perforation tests, & Urease tests were performed to differentiate species.

Slide culture: The pattern of growth, morphology of hyphae, shape and size of macroconidia, and microconidia were observed under microscope.[1]

Hair perforation test: It was performed to differentiate between *T.rubrum* & *T.metagrophyte*. Isolates of *T.mentagrophytes* produced marked wedge shaped perforations in hairs whereas isolates of *T.rubrum* did not produce any perforation in hairs. This is because *T.mentagrophyte* produces enzyme that penetrates hair and produces conical (wedge shaped) perforation in human hairs.(1)

Urease test: Small amount of culture colony from slopes of SDCCA was inoculated on Christensen's urea agar slope and was incubated at room temperature for 7 days. A change in colour from yellow to pink was considered as positive.

Finally, the identification of causative fungus was done from the colony characteristics on SDCCA, rate of growth, microscopic features and results of various specific tests.(1)

Stastistical Methods

- Data was compiled in MS-EXCEL sheet.
- Statistical package for social sciences (SPSS), was used for data analysis.
- Chi – square test was used to check association between different attributes.

RESULTS

In this study 138 samples from clinically suspected cases of dermatophytoses were taken, the skin specimens were of 86 (62.31%), the nail specimens were of 45 (32.60%), and the hair specimen were of 07 (5.07%). Growth was observed in 61(44.2%) cases, in which 46 (75.40%) were of dermatophytes, 15 (24.59%) were of non dermatophytic isolates.

Table 1: Age and sex distribution of patients (n=138)

Age groups (years)	Male	Female	Total
0-10	01 (0.72%)	02 (1.44%)	03 (2.17%)
11-20	10 (7.24%)	03 (2.17%)	13 (9.42%)
21-30	23 (16.66%)	19 (13.76%)	42 (30.43%)
31-40	17 (12.31%)	12 (8.69%)	29 (21.01%)
41-50	10 (7.24%)	07 (5.07%)	17 (12.31%)
Above 50 years	22 (15.94%)	12 (8.69%)	34 (24.63%)
Total	83 (60.14%)	55 (39.85%)	138 (100%)

The male to female ratio is 1.5:1. The predominant age group in males and females was 21-30 (30.43%) years.

Table 2: Risk factors associated with culture positive dermatophytosis cases (n=46)

Risk factors or associated conditions	No of patients
Contact with Pets	12 (26.08%)
Swimming	10 (21.73%)
Contact with patients with similar lesions	10 (21.73%)
Sports	08 (17.39%)
Diabetes	04 (8.6%)
Thyroid disorder	02 (4.3%)
Total	46 (100%)

In our study, the most common risk factor to cause dermatophytosis was contact with pets and was found in 12 (26.08%) cases. In 10 (21.73%) cases each it was found to have contact with patients having same lesions of either family members or friends and swimming. Other risk factors were sports, diabetes and thyroid disorder which played significant role in causing disorder.

Table 3: Fungal agent isolated from various clinical samples from suspected cases of dermatophytosis (total = 61)

Isolate	Skin	Hair	Nail	Total
Trichophyton mentagrophytes	17 (27.86%)	00 (00%)	06 (9.83%)	23 (37.70%)
Trichophyton rubrum	11 (18.03%)	02 (3.27%)	07 (11.47%)	20 (32.78%)
Trichophyton tonsurans	01 (1.63%)	00 (00%)	00 (00%)	01 (1.63%)
Microsporum gypseum	02 (3.27%)	00 (00%)	00 (00%)	02 (3.27%)
Aspergillus niger	04 (6.55%)	00 (00%)	06 (9.83%)	10 (16.39%)
Candida species	01 (1.63%)	00 (00%)	03 (4.91%)	04 (6.55%)
Aspergillus flavus	00 (00%)	00 (00%)	01 (1.63%)	01 (1.63%)
Total	36 (59.01%)	02 (3.27%)	23 (37.70%)	61 (100%)

In our study, dermatophytic isolates were 46 (75.40%), and non dermatophytic molds were 11(18.03%), and candida species were 4 (6.55%). Most common pathogenic dermatophyte isolate in our study was T. mentagrophytes (50%), followed by T. rubrum (43.47%), M. gypseum (4.3%) and T. tonsurans (2.1%). Dermatophytosis constituted about 33.3% % of all superficial skin diseases.

We found candida species in four cases and non dermatophytic isolates from eleven cases. Non dermatophytic molds isolated in this study were A.niger and A. flavus. Here we did not found any association between type of isolate and sample type. (p value – 0.559 NS).

Table 4: Clinical patterns of suspected cases of dermatophytosis and their species (n=138)

Clinical pattern	*T.mentagrophyte*	*T.rubrum*	*T.tonsurans*	*M.gypseum*	No dermatophyte fungus isolated	Total no of patients
T.corporis	11 (7.9%)	02 (1.44%)	01 (0.72%)	01 (0.72%)	25 (18.11%)	40 (28.98%)
T.cruris	04 (2.89%)	07 (5.02%)	00 (00%)	01 (0.72%)	19 (13.76%)	31 (22.46%)
T.pedis	00 (00%)	01 (0.72%)	00 (00%)	00 (00%)	05 (3.62%)	06 (4.34%)
T.facium	01 (0.72%)	01 (0.72%)	00 (00%)	00 (00%)	05 (3.62%)	07 (5.07%)
T.mannum	01 (0.72%)	00 (00%)	00 (00%)	00 (00%)	01 (0.72%)	02 (1.44%)
T.capitis	00 (00%)	02 (1.44%)	00 (00%)	00 (00%)	05 (3.62%)	07 (5.07%)
T.unguium	06 (4.34%)	07 (5.07%)	00 (00%)	00 (00%)	32 (23.18%)	45 (32.60%)
Total	23 (16.66%)	20 (14.49%)	01 (0.72%)	02 (1.44%)	92 (66.66%)	138 (100%)

In this study, Tinea Unguium (32.6%) was the common clinical pattern followed by Tinea Corporis (28.98%) and Tinea Cruris (22.46%).

Table 5: Result of KOH and DMSO mount and type of specimen (n=138)

KOH and DMSO result	Skin	Hair	Nail	Total (%)	Chi square	P value
Positive	43 (31.15%)	03 (2.17%)	27 (19.56%)	73 (52.89%)	1.48	0.476 (NS)
Negative	43 (31.15%)	04 (2.89%)	18 (13.04%)	65 (47.10%)		
Total (%)	86 (62.31%)	07 (5.07%)	45 (32.60%)	138 (100%)		

The 52.89% patients were found to be KOH and DMSO positive, while the remaining 47.10% were found negative. There is no association between KOH and DMSO positivity with the type of specimen. (p value – 0.476 NS).

Table 6: Culture positivity and type of specimen (n=138)

Results of culture	Skin	Hair	Nail	Total (%)	Chi square	P value
Positive	31 (22.46%)	02 (1.44%)	13 (9.42%)	46 (33.33%)	0.756	0.685 (NS)
Negative	55 (39.85%)	05 (3.62%)	32 (23.18%)	92 (66.66%)		
Total (%)	86 (62.31%)	07 (5.07%)	45 (32.60%)	138 (100%)		

Out of 138 cases 61 (44.20%) cases were positive by culture and 77 (55.79%) were negative by culture. There is no association between culture positivity with the type of specimen. (p value – 0.685 NS).

Table 7: Correlation between (KOH + DMSO) microscopy and culture (n=138)

	KOH and DMSO positive	KOH and DMSO negative	Total	Chi square	P value
Culture positive	60 (98.36%)	01 (1.63%)	61 (100%)	55.9	0.0001 (S)
Culture negative	13 (31.16%)	64 (68.83%)	77 (100%)		
Total	73 (60.86%)	65 (39.13%)	138 (100%)		

In this study there is a good correlation between microscopy and culture, hence both the things are very important for diagnosis.

DISCUSSION

Clinically suspected 138 cases of dermatophytosis attending the skin and venereal disease outpatient department were included in the present study. Cases of all the age group and both sexes were included, detailed history, clinical examination findings and relevant investigations of patients were recorded.

Age and sex distribution of patients

In our study, out of 138 cases, 83 (60.14%) were males and 55 (39.85%) were females. The male to female ratio is 1.5:1. Our findings are in accordance with Sabyasachi Banerjee et al,[9] Kennedy Kumar et al,[12] P.V.Doddamani et al,[14] Dr. NIlekar et al,[10] Gupta C M et al,[17] Hemangi Walke et al,[15] and Amodkumar Yadav et al.[3]

The male predominance may be due to their more exposure to an environment of greater outdoor

activity and increased sweating providing favorable environment for fungi to grow and females may hide their diseases in covered parts of the body.

In our study, the predominant age group was 21-30 years. Same observations were also supported by other workers such as Sabyasachi Banerjee et al,[9] Kennedy Kumar et al,[12] P.V.Doddamani et al,[14] Dr. NIlekar et al,[10] Hemangi Walke et al,[15] and Amodkumar Yadav et al,[3] Clarrisa J Lygdoh et al,[2] except Gupta C.M et al,[17] they observed predominant age group was above 60 years of age.

This highest incidence in this age group may be due to increased physical activity and increased chances of exposure to infection.

Clinical patterns of dermatophytosis

In this study, Tinea Unguium (32.6%) was the common clinical pattern followed by Tinea Corporis (28.98%) and Tinea Cruris (22.46%). This finding is comparable with the previous study conducted by Gupta C.M et al,[17] who observed T.unguium (52%) as the common clinical pattern followed by T. corporis (25%). However other workers Sabyasachi Banerjee et al,[9] Kennedy Kumar et al,[12] Dr. NIlekar et al,[10] Hemangi Walke et al,[15] and Amodkumar Yadav et al,[3] Clarrisa J Lygdoh et al,[2] Amita Pandey et al,[16] Matnani G et al,[11] and Soumya Nassimuddin et al,[19] found Tonea corporis as the common clinical pattern. This higher incidence of Tinea unguium may be affected by geographical distribution and the patient class attending the outpatient department. As agricultural workers are second most common group in our study that favors nail infection.

Most common dermatophyte isolate in our study was T. mentagrophytes (50%), followed by T. rubrum (43.47%), M. gypseum (4.3%) and T. tonsurans (2.1%). Our finding coincides with the findings of Soumya Nasimuddin et al, they also found T. mentagrophyte (38.75%) common isolate. But our this finding is not in accordance with Sabyasachi Banerjee et al,[9] Kennedy Kumar et al,[12] Dr. NIlekar et al,[10] Hemangi Walke et al,[15] and Amodkumar Yadav et al,[3] Clarrisa J Lygdoh et al,[2] Amita Pandey et al,[16] Matnani G et al,[11] Gupta C M et al,[17] and P.V.Doddamani et al.[14] They all found T. rubrum was the most common species. Reason for more prevalence of T. mentagrophyte in our study may be due geographical difference and history of contact with pet animals; may be the contributing factor and causing infections which may be acquired from pet animals.

In our study, the most common risk factor to dermatophytosis was contact with pets and was found in 12 (26.08%) cases. In 10 (21.73%) cases each it was found to have contact with patients having same lesions of either family members or friends and association of swimming. Other risk factors were sports, diabetes, thyroid disorder which plays significant role in causing disorder. Clarrisa J Lygdoh et al,[2] reported that 10.3% cases had animal contact history. Gupta C M et al,[17] reported that patients having close association with domestic or pet animals such as cattle, dogs, cats and fowl had predominance of dermatophytic infections. Prachala G R,[13] reported contact with infected family members in 21 (14%) cases and contact with animals in 27 (18%) cases.

In our study, we found that majority of patients were housewives (22.46%) followed by agricultural workers (19.56%). Gupta C M et al,[17] reported dermatophytosis was predominant in lower economic groups such as farmers and daily wage workers or laborers. Hemangi Walke et al,[15] reported that dermatophytosis was most commonly seen in manual workers (39.87%) including farmers, labourers, butchers, and carpenters. In our study, the housewives are more commonly affected that may be due to increased wet work in housewives. Prachala G R,[13] revealed that higher number of cases were 79 (52.67%) belonged from labour class followed by households 36 (24%)

In our study, the 52.89% patients were found to be KOH and DMSO positive, while the remaining 47.10% were found negative. Dermatophytosis constituted about 33.3% % of all superficial skin diseases.

CONCLUSION

In this study clinically suspected 138 cases of dermatophytoses were included. Tinea Unguium was the commonest clinical pattern found followed by Tinea Corporis. Trichophyton mentagrophytes was the predominant isolate from patients with Tinea Corporis & Trichophyton rubrum was the predominant isolate from the patients with Tinea cruris.

Out of 61 positive specimens for culture, 60 were positive by both culture and microscopy and only one was positive by culture which was negative by microscopy, while out of 77 samples which were culture negative, 13 were culture negative but microscopy positive and 64 were negative by both culture and microscopy. That means there is very good correlation between microscopy and culture positivity. Hence microscopic examinations as well as culture both are equally important for the diagnosis of dermatophytosis.

Higher incidence was noted amongst males than females and the ratio being 1.5:1.

Dermatophytosis was found commonest in housewives followed by agricultural workers. Common risk conditions are household works, agricultural works, outside work with increased sweating, wet work and increased contact with soil leads to humid and moist areas favoring sites for dermatophytic infections; all these factors predispose dermatophytic infections.

In our study, the most common risk factor to dermatophytosis was contact with pets and contact with patients having same lesions of either family members or friends. Common risk factors found are contact with pets & patients or family members with

dermatophytosis; swimming in common pools and sports.

We should give some advice to the persons affected by dermatophytic infections to avoid sharing of personal belongings like towel, comb or clothes; also avoid swimming in common pools till having the dermatophytic infections. Look for the pet contact and the pets should also be treated for the same if infected. The persons working in wet areas or in soil should take bath after their completion of work. All these things will help in preventing the dermatophytic infections and further transmission.

Ethical Consideration

Approval from the Institutional Ethics Committee of MGM Medical College and Hospital, Auranbagad, MGMIHS Navi Mumbai, Maharashtra, India

(Letter no – MGM-ECRHS /2016/08, Dated-25/11/2016)

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