International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 5, May 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.5.2025.76

ORIGINAL RESEARCH

Clinico-Mycological Characterization of fungal isolates from cutaneous skin lesions

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Received: 23 March, 2025 Accepted: 30 April, 2025 Published: 10 May, 2025

ABSTRACT

Aims and Objectives: Superficial cutaneous mycoses are commonly encountered fungal diseases prevalent in most parts of the world involving the skin's outermost covering as well as its appendages such as nail and hair. This study was carried out to study the correlation between clinical features and the fungal isolates obtained. **Study design:** Observational (prospective) study. **Place and Duration of Study:**Department of Microbiology and Department of Dermatology, Gandhi Medical College and Hamidia Hospital Bhopal, Madhya Pradesh, India, between January2020 to October 2021. **Methodology:** This study was conducted in Department of Microbiology, Gandhi Medical College, Hamidia and Associated hospitals, Bhopal (M.P.) from January 2020 to October 2021. A total of 180 samples were collected from patients suspected of superficial mycoses which were then subjected to direct KOH examination and culture on plain SDA, on SDA with antibiotics slants (cycloheximide and chloramphenicol), on Dermatophyte Test Medium and in cases of suspected lipophilic fungi, samples were inoculated on SDA with olive oil overlay. The growths were examined macroscopically as well as microscopically. Germ tube tests and inoculations on CHROMagar Candida Medium were performed on all growths identified as yeasts.
Results: In our study, majority of patients, belonged to the age group of 21-30 years (25%) with a male to female ratio of 2:1. Tinea corporis (27.2%) was the most common clinical types. KOH mount was found positive in 74 (41.1%) and culture was positive in 76 (42.2%) samples. Trichophytonmentagrophytes (32.89%) was the predominant fungal pathogen isolated.
Conclusion: The diagnostic yields of KOH mount and culture were found to be mutually beneficial. As a result, both approaches, when combined with clinical evidence, are equally important in determining superficial mycosis.

Keywords: Non albicans Candida, Germ tube test, CHROMagar, Dermatophyte Test Medium, Tinea corporis, Trichophytonmentagrophytes

INTRODUCTION

Superficial cutaneous mycoses are the fungal infections involving the outermost skin's covering as well as its appendages such as nail and hair. They are prevalent worldwide and are believed to affect 20% to 25% of the world's population and their incidence are on a continuous rise (1). They are classified into two broad groups; the "Surface infections" which constitute Pityriasis versicolor, Tinea nigra and Piedra. And the "Cutaneous infections" including Dermatophytoses and Candidiasis (2).

In surface infections, as there is no contact of fungi with the living tissue, so there will be absence of any inflammatory response because in this fungi live exclusively on the skin's dead layers. In contrast, cutaneous infections are usually restricted to the skin's cornified layer and its appendages and are associated with inflammatory and allergic responses (2).

Pityriasis versicolor (tinea versicolor) is a common, recurrent, superficial fungal infection of stratum corneum which is caused by Malassezia furfur. Tinea nigra is marked by brownish black coloured, non-scaly macular patches that typically affect the palms, soles, and hardly other regions of the body (3). Piedra is a mycotic infection affecting the hair shaft characterised by the formation of solid, irregular nodules formed of fungal components that are adhered to the hair shaft (4).

Dermatophytoses are responsible for most superficial fungal infections and are very common throughout the world. These are caused by a group of strongly associated keratinophilic fungi, quite efficient of penetrating keratinized skin tissues and appendages such as hair and nails. Dermatomycoses are skin infections caused by non-dermatophytic fungus, whereas piedra and onychomycoses are hair and nail

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infections, respectively (5). Dermatophytes are hyaline septate moulds that are classified into three genera based on their morphological traits; Trichophyton, Microsporum and Epidermophyton. These three genera comprises several different species (6). The contributing factors in causation of superficial cutaneous mycosis are warm moist climate, maceration, minor skin trauma and occlusion, immunodeficient conditions, poor sanitation (7).

Clinically, tinea can be classified depending on the site of involvement including Tinea capitis (scalp), Tinea barbae (bearded regions of neck and face), T.corporis (hairless skin of the body), Tinea cruris (groin), T.faciei (face), Tinea manuum (palms), Tinea pedis (athlete's foot), Tinea unguuim (nails), Tinea imbricate (concentric rings of papulosquamous scaly patches) (8). Candidiasis is an infection caused by Candida spp. that can range from superficial skin infections and mucosal membrane infections to a systemic and potentially fatal condition (9).

Although superficial fungal infections are primarily identified clinically, they frequently resemble other skin infections as a consequence of specific drugs, such as topical steroid ointments and creams, leading to inaccurate diagnosis and management. As a result, laboratory testing of these superficial fungal infections is required in order to diagnose them quickly, reliably, and efficiently. This study was carried out to determine clinical patterns of various types of cutaneous fungal infections and to study the correlation between clinical features and the fungal isolates obtained.

MATERIAL AND METHODS

This prospective observational study was conducted in Department of Microbiology, Gandhi Medical College, Hamidia and Associated hospitals Bhopal, Madhya Pradesh. Samples from patients suspected of superficial cutaneous mycoses in the Department of Dermatology, were collected and processed for mycological evaluation in the State Virology Laboratory, Department of Microbiology, Gandhi Medical College, Hamidia hospital, Bhopal. A total of 180 samples received in Department of Microbiology during the study period from January 2020 to October 2021 were recruited in the study.

History and other relevant data was collected from patients or from their attenders and also from physicians and requisition forms from patients. Informed written consent was obtained from all the patients. After proper history taking and physical examination of the patients, samples were collected adequately and precisely with all aseptic precautions. The samples were harvested in a sufficient amount and taken from the edge of the infected area, which corresponds to the active zone of the lesion. The following materials were used for isolation of dermatophytes: skin, nail and hair. The collected specimens were divided into two portions. The first portion of the specimens was examined microscopically using 10-20% (hair and skin scrappings) potassium hydroxide (KOH). Nail clippings were immersed in 40% KOH overnight and examined next morning.

The second portion was inoculated on plain Sabouraud's Dextrose Agar (SDA) and SDA with antibiotics slants (cycloheximide and chloramphenicol) and in cases of suspected lipophilic fungi samples were inoculated on SDA with olive oil overlay. All cultures were examined bi-weekly for growth and incubated for maximum 6 weeks before declaring them negative. Samples were also inoculated on Dermatophyte Test Medium for the confirmation of dermatophytes. The growths were noted for colony characteristics in the form of texture, surface, colour on the obverse and reverse and any diffusible pigment. Lactophenol Cotton Blue mount, and Slide cultures were undertaken for microscopic morphology. Germ tube tests and inoculations on CHROMagar Candida Medium were performed on all growths identified as yeasts.

RESULTS

A total of 180 patients of suspected superficial cutaneous fungal infections were enrolled in the study comprising of 120 (66.7%) males and 60 (33.3%) females, showing a male preponderance. Overall male to female ratio was 2:1. The majority of the patients were from the age group 21-30 years (25%), followed by 11-20 years (19.4%) and 31-40 years (19.4%) and least from the age group 61-70 years (1.7%). Students (38.9%), labourers (23.3%) and farmers (13.2%) dominated the study group. The majority of cases were found in July (23.3%), followed by August (12.8%) and November 23 (12.8%).

Most common type of clinical manifestation found was Tinea corporis 49 (27.2%), followed by Onychomycosis 28 (15.6%), Tinea capitis 27 (15%), Tinea manuum 23 (12.8%), Tinea pedis 21 (11.7%), Tinea cruris 9 (5%), Tinea faciei 7 (3.9%), Pruritus 5 (2.7%), Tinea barbae 3 (1.7%), Intertrigo 3 (1.7%), Tinea versicolor 3 (1.7%) and Eczema 2 (1.1%). Poor personal hygiene was the most common risk factor in majority of the patients (42.2%), followed by topical steroid use (20%) and diabetes mellitus (20%).

Out of 180 samples, 74 (41.1%) samples were KOH positive, out of which 36 (48.65%) samples were culture positive. 106 (58.9%) samples were KOH negative, out of which 40 (37.74%) samples were culture positive. Hence, in present study, culture positivity was 76 (42.2%) samples. Whereas 66 (62.26%) samples were both culture and KOH negative. (Table 1 & Figure 1)

In our study, Trichophytonmentagrophytes (32.89%) was found to be the most common fungal pathogen isolated followed by Candida tropicalis (27.63%), Candida albicans (23.69%), Candida krusei (7.9%), Trichophytonrubrum (5.26%), and Candida glabrata (2.63%). (Table 2 & Figure 2)

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Table 1 - Correlation between KOH mount and culture findings

	Culture positive (76)	Culture negative (104)
KOH positive (74)	36 (48.65%)	38 (51.35%)
KOH negative (106)	40 (37.74%)	66 (62.26%)

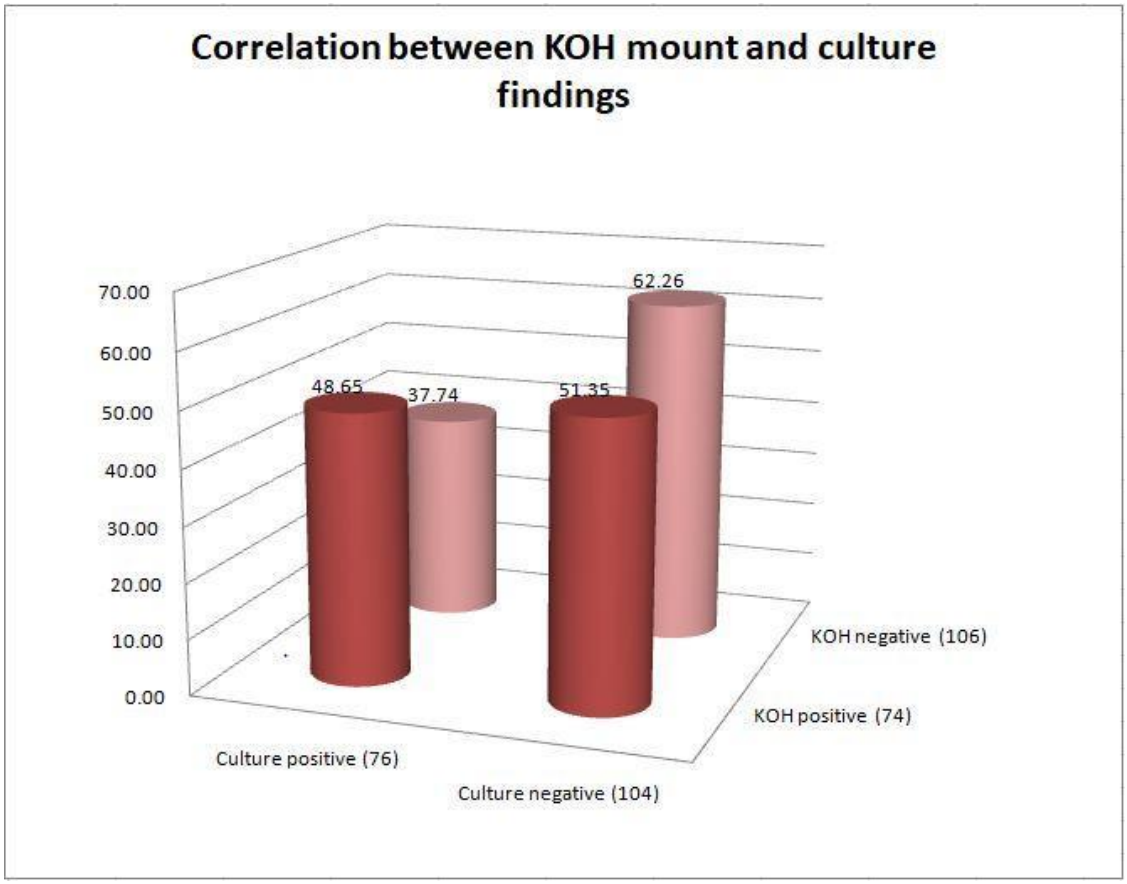

Figure1: Correlation between KOH mount and culture findings

Table 2 - Correlation between species of fungi isolated on culture and type of sample taken

Isolates on culture	Skin scrapping	Hair clipping	Nail clipping	Total (n=76)
Candida albicans	15	2	1	18
Candida glabrata	2	0	0	2
Candida krusei	3	2	1	6
Candida tropicalis	14	4	3	21
Trichophytonmentagrophytes	14	5	6	25
Trichophytonrubrum	2	1	1	4

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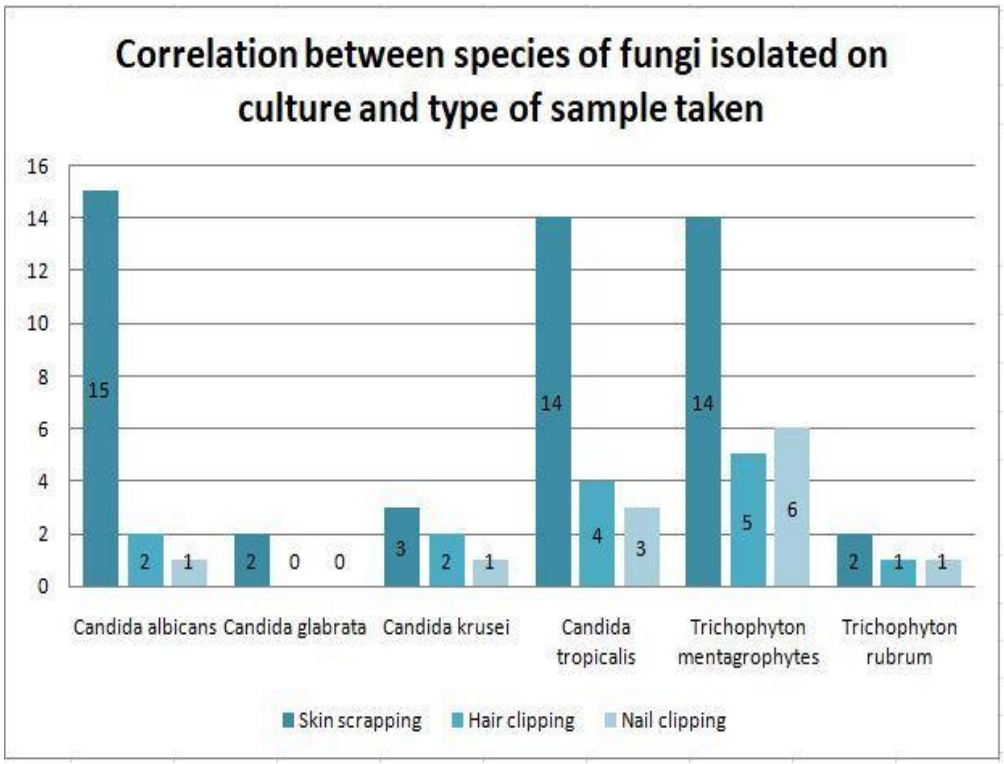

Figure 2: Correlation between species of fungi isolated on culture and type of sample taken

DISCUSSION

Superficial mycosis forms a large fraction of ailments in patients attending the skin OPD of our centre. In our study, **42.2%** cases clinically suspected of superficial cutaneous mycosis were confirmed by laboratory diagnosis. Whereas, according to **Sharma B et al. (10)**, the prevalence of superficial cutaneous mycosis in Andhra Pradesh is 33.61% in Amritsar (62.3%), in Bihar (44.26%), in Chennai (85%), in Dehradun (24.13%), in Gujrat (35.26%), in Hyderabad (58.6%), in Jammu and Kashmir (41.8%), in Karnataka (48.3%), in Lucknow (37%), in Manipur (60%), in North-east India (20.5%), in Tamil Nadu (22.9%), in Telangana (68%), in Uttar Pradesh (17.77%), in Uttarakhand (37.5%) and in Central India (68.34%).

Amongst all the patients suspected of superficial cutaneous mycosis, maximum number of patients were found in the age group 21-30 years of age (25%), followed by 11-20 years (19.4%). The high prevalence of these infections within this age group may be attributed to various factors, such as increased physical activity, changes in the hormonal pattern and increased opportunity for exposure. In a similar study by **Grover et al., (11)**more than two-thirds of the patients were between the age group of 21 and 40 years, which is the most common age group for outdoor physical activity. However, **Binduet al.,(12)** observed a higher prevalence in the age group of 11-20 years. One of the possible reasons why prepubescent children are more susceptible to these infections is an insufficient amount of fungi-inhibiting fatty acids.

In the present study, males (66.7%) were more commonly affected than females (33.3%). Male to female ratio was 2:1, which was similar to studies by **Hazarika D et al. (13), Noronha TM et al. (14), Singh S et al. (15), Hosthota A et al. (16),** and **Pradhan MB et al. (17)**. Males are more likely to contract these illnesses due to their increased exposure to outdoor activities such as sports and other jobs that require higher physical activity. Females, on the other hand, have a lower incidence, which could be owing to their lifestyle (more housework and indoor activities) as well as hormonal considerations, as the female hormone progesterone has been identified as an efficient inhibitor of fungal development. Furthermore, because of the prevalent social stigma in India's rural population, female cases may not be reported to hospitals, resulting in a lower prevalence in females.

However, **Belurkar et al. (18)**, differed in showing female preponderance with male to female ratio being 0.6:1. This may be due to females are more likely to be exposed to humid or wet atmosphere at home (particularly in the kitchen), which is conducive to the spread of fungal diseases. Due to societal considerations, adult females wear more clothing than their male counterparts, especially in hot and humid weather. Sweating is more common among people who work in manual labour. Female hormonal shifts have also been linked to the development of dermatophytoses.

In our study, the occupation-wise analysis showed that the students, (38.9%), labourers (23.3%) and farmers (13.2%) were mostly affected. The higher

DOI: 10.69605/ijlbpr_14.5.2025.76

prevalence among students can be attributed to their careless attitude towards cleanliness and hygiene, sports activities, increased freedom of movement, lack of personal hygiene guidance, changing fashion trends, and preference for tight, ill-fitting dresses. The common occurrence of these infections in farmers and labourers may be because the nature of their profession requires them to sweat excessively owing to long periods of exposure to the sun, harmful fungus thrive even more.In our present study, majority of cases were found in July (23.3%), followed by August (12.8%) and November (12.8%). This finding is in concordance with the studies carried out by **Verma S et al.(19)**, **Kaur I et al.(20),** and **Shah A et al.(21).** This is because monsoon season and the humid climate favours the fungal proliferation.

We found that tinea corporis (27.2%) was the most common clinical variety which is in corroboration with the studies carried out by **Grover et al.(11), Hazarika D et al.(13), Sen SS et al.(22), Noronha TM et al. (14), Pradhan MB et al. (17)** and**Binduet al.,(12).** On the contrary, **Gupta CM et al. (23),** in his study observed tinea unguium as the commonest clinical variety. The higher frequency of tinea unguium could be due to the fact that the infection is usually asymptomatic, and the elderly are less likely to seek medical help. Infected nails act as a long-term reservoir of infection, allowing for recurrent mycotic infections of the skin. The variations in the clinical type of superficial cutaneous mycosis could be related to a variety of factors such as climatic circumstances, population migration to earn a livelihood, type of occupation, and pathogen-host relationship.

In the present study, poor hygiene was observed in 42.2% of patients, indicating that it was an essential factor observed among our subjects, which is in congruent with the studies by **Hosthota A et al. (16), Sharma M et al. (24)** and **Ranganathan S et al. (25).**Living conditions, large family size and close contact, either directly or by sharing facilities, including combs and towels, is common between family members in low socioeconomic strata people which may facilitate transmission. Direct microscopy with KOH was positive in **41.1%** of samples of suspected superficial cutaneous mycosis which is nearly consistent with the study done by **Khadka S et al. (5)** in which the KOH positivity rate was **44.5%.** Similar findings were observed by **Kaur I et al.(20), Mohanty JC et al.(26)** and **Sen SS et al.(22)** where the KOH positivity rates were **48%, 48.4%** and **49%** respectively.

On the contrary to this, higher KOH positivity rates were observed by **Munir S etal. (27)** and **Karmakar S et al. (28)** that is **74%** and **88.4%** respectively. The KOH positivity rate ranged from 35.6% to 88.6% in several studies.

In the present study, culture was found positive in **42.2%** of samples of suspected superficial cutaneous mycosis which is in congruent with the studies done by **Shah A et al. (21), Singh S et al.(15), Gupta CM et al.(23), Karmakar S et al.(26),Noronha TM et al. (14), and Sharma M et al.(24)** where the culture positivity rates were **41.6%, 44.62%, 46%, 41.6%,40%,** and**50.8%** respectively.

On the contrary to this, higher culture positivity rate was observed by **Belurkar et al. (18)** and **Grover et al., (11)** that is **71%** and **79.1%** respectively. A very low culture positivity rate was observed by **BhavsarHitendra K et al.(29)** that is **20.15%.** In various studies, the culture positivity rate varied from 36% to 53.6%.

The variation in the KOH and culture positivity rates may be attributed to case selection criteria, skill involved in sampling technique and expertise required in performing the procedure. Non visualization of hyphae on direct microscopy could be due to a significant inflammmatory response which obscures them or because of improper digestion of cellular and keratin debris due to longer time period required for their digestion. Also the rate of fungal isolation is very much dependent on good and appropriate culture techniques as well as proper aseptic precautions, which prevent contaminants to overgrow in the culture. (28)

In present study, 36 (48.65%) samples were both KOH and culture positive, 38 (51.35%) samples were KOH positive but culture negative, 40 (37.74%) samples were culture positive but KOH negative and 66 (62.26%) samples were both culture and KOH negative. These results highlight the importance of both culture as well as KOH mount for accurate diagnosis of superficial mycoses.

Among the culture positive samples, **Trichophytonmentagrophytes (32.89%)** was the most common fungal pathogen isolated followed by Candida tropicalis (27.63%), Candida albicans (23.69%), Candida krusei (7.9%), Trichophytonrubrum (5.26%), and Candida glabrata (2.63%).

The findings of our study correlated with the studies by **Khadka S et al. (5), Ganesan K et al.(30), Verma S et al.(31), Shah A et al.(21), Bhatia VK et al.(32)** and **Putta SD et al.(33)**that showed **Trichophytonmentagrophyte** as the most common isolate. However, **Trichophytonrubrum** was reported as the most prevalent isolate by **Patel P et al.(34), Malik A et al.(1), Nawal P et al.(2), Singh Set al.(15)** and **Hazarika D et al.(13).** This could be due to differences in the environmental condition and variations in the distribution of different dermatophyte species in different parts of our country.

CONCLUSION

In conclusion, the present study of 180 cases at our tertiary care hospital, shows that males are predominantly affected with preponderance of cases in the monsoon months. The diagnostic yields of KOH mount and culture were found to be mutually beneficial. Majority of the cases were from T. corporis and most common etiological agent is

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Trichophytonmentagrophyte. Although the findings of this study matches with many studies done across India, it differs significantly with some studies suggesting the role of geographical variation in clinical and mycological pattern.

Despite the fact that the clinical diagnosis is the cornerstone of superficial fungal infections, any clinical diagnosis must be backed up by laboratory evidence because antifungal therapy based on the proper laboratory confirmation and identification of fungal isolates would be more effective in the treatment of such superficial mycotic infections.

LIMITATIONS

As this study had a relatively smaller sample size, the results may vary with a large study group.However, long-term studies with a larger sample size are needed to comment on the current scenario of superficial mycosis. Also no antifungal susceptibility testing of any of the isolate was performed.In addition, other diagnostic modalities such as Calcofluor White staining may have to be done to further improve the recovery of fungi from suspected cases of superficial fungal infections.

ACKNOWLEDGEMENTS

The authors would like to acknowledge all the staff of Department of Dermatology and Department of Microbiology at Gandhi Medical College, Bhopal (M.P.).

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

NR - designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. JL - managed the analyses of the study, concept coordination and preparation of the manuscript. PS – revision of manuscript. DC - managed the literature searches. All authors read and approved the final manuscript.

CONSENT

All authors declare that an informed written consent was obtained from the patients.

ETHICAL APPROVAL

Our study was approved by the Institutional Ethics Committee of Gandhi Medical College and Hamidia Hospital, Bhopal (M.P.)

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