



Clinico-Microbiological Profile and Antifungal Susceptibility of Subcutaneous Mycoses with Bone Involvement: A Cross-Sectional Study from a Tertiary Care Center in Eastern Odisha, India.

¹Sajid Khan, ^{*2}Sasmita Khatua, ³Madhumita Swain.

¹Senior resident, Department of Microbiology, Fakir Mohan Medical College, Balasore, Odisha, India

²Associate Professor, Department of Microbiology, Pandit Raghunath Murmu Medical College, Baripada, Odisha, India

³Assistant Professor, Department of Microbiology, DDMCH, Keonjhar, Odisha, India

Abstract

Background:

Subcutaneous mycoses are chronic fungal infections affecting the skin and subcutaneous tissues, commonly occurring in tropical regions among individuals engaged in outdoor occupations. Accurate identification and antifungal susceptibility testing are important for appropriate management due to emerging drug resistance.

Objective:

To determine the prevalence of subcutaneous mycoses, identify the causative fungal species, and evaluate antifungal susceptibility patterns among clinically suspected cases.

Methods:

This cross-sectional study was conducted from September 2021 to August 2023 among patients clinically suspected of subcutaneous mycoses attending a tertiary care center in Eastern Odisha, India. Clinical samples, including skin scrapings, tissue, pus, discharge, granules, and biopsy specimens, were collected aseptically. Samples were examined using a potassium hydroxide (KOH) mount, cultured on Sabouraud dextrose agar, and identified using standard microbiological techniques. Histopathological examination was performed using hematoxylin and eosin staining. Antifungal susceptibility testing was conducted using the microbroth dilution method.

Results:

Among 110 clinically suspected cases, subcutaneous mycoses were detected in 52.72% by culture, 78.18% by KOH mount, and 80% by histopathology. The most common isolates were *Madurella* spp. (48.27%), followed by *Exophiala* spp. (29.31%) and *Fonsecaea* spp. (22.41%). All isolates showed resistance to fluconazole and anidulafungin, while most isolates were sensitive to itraconazole, posaconazole, and amphotericin B.

Conclusion:

Subcutaneous mycoses remain prevalent in rural populations with occupational exposure. Antifungal susceptibility testing is essential for guiding therapy, with itraconazole, posaconazole, and amphotericin B demonstrating better efficacy compared to fluconazole and anidulafungin.

Keywords: Subcutaneous mycoses, antifungal susceptibility, histopathology, fungal culture.

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Corresponding author: Sasmita Khatua

Email: drkhatua.sasmita@gmail.com

Associate Professor, Department of Microbiology, Pandit Raghunath Murmu Medical College, Baripada, Odisha, India.

Introduction

Subcutaneous mycoses, or implantation mycoses, result from fungi entering subcutaneous tissue via injury, prevalent in humid, tropical areas among low socio-economic groups.[1] Global warming has fostered the prevalence of thermo-tolerant fungal species in mammals, potentially increasing pathogenicity.[2] Around 400 fungal species are pathogenic to humans, with new ones

emerging annually, showing variable incidence across continents, especially within specific latitudes.[3] In India, regions like the sub-Himalayas and coastal areas are endemic for chromoblastomycosis, affecting rural males engaged in outdoor activities. Lesions typically manifest as localized papules, nodules, or plaques, with rare severe clinical forms or dissemination.[4-5] Types of mycoses include sporotrichosis, mycetoma, chromoblastomycosis,



phaeohyphomycosis, and rhinosporidiosis caused by various fungi.[6]

Histopathological confirmation is crucial for diagnosis, but misdiagnosis is common, posing therapeutic challenges.[7] Delayed or misdirected diagnosis contributes to the difficulty in treating chronic conditions like chromoblastomycosis and Madura foot. Histopathology remains the principal investigation despite potential discrepancies with culture results. Subcutaneous mycoses are developing resistance to antifungal drugs, necessitating continuous improvement in identification and susceptibility testing.[8-10] As a part of hospital prevention and control, and to track antifungal susceptibility to different fungi and to guide clinicians in our setup, this study was conducted with the following objectives. (a) Prevalence of cases of subcutaneous mycoses (b) Identification of various species causing subcutaneous mycoses (c) To do the antifungal susceptibility test of those isolated fungi.

Materials & methods

Study Design

A **hospital-based cross-sectional study** was conducted to evaluate the clinico-microbiological profile and antifungal susceptibility patterns of subcutaneous mycoses.

Study Setting

The study was carried out in the Department of Microbiology at a tertiary care hospital in Eastern Odisha, India, from **September 2021 to August 2023**.

Study Participants

Patients of all age groups and genders presenting with **clinically suspected subcutaneous mycoses** were considered for inclusion.

Inclusion Criteria

Patients with clinically suspected subcutaneous mycoses
Patients willing to provide informed consent

Exclusion Criteria

Patients diagnosed with fungal infections other than subcutaneous mycoses
Patients unwilling to participate in the study

Sampling Procedure

Clinical samples, including skin scrapings, tissue, pus, discharge, granules, and biopsy specimens, were collected aseptically from suspected lesions.

Data Collection

Demographic data, clinical history, occupation, and socio-economic status were recorded using a structured proforma. Detailed clinical examination, including site, size, and number of lesions, was performed.

Laboratory Procedures

Samples were examined by a **10–20% potassium hydroxide (KOH) mount** for direct microscopy. Cultures were performed on **Sabouraud dextrose agar (SDA)** with and without cycloheximide and incubated at **25°C and 37°C**. Fungal isolates were identified based on colony morphology and microscopic features using lactophenol cotton blue (LPCB) mount and slide culture techniques.

Histopathological examination using **hematoxylin and eosin staining** was conducted to identify fungal elements and associated tissue reactions.

Antifungal Susceptibility Testing

Antifungal susceptibility testing was performed using the **microbroth dilution method** against the following antifungal agents: fluconazole, anidulafungin, itraconazole, posaconazole, and amphotericin B.

Study Variables

The main variables included demographic characteristics, clinical parameters, fungal species isolated, diagnostic test results, and antifungal susceptibility patterns.

Statistical Analysis

Data were entered in **Microsoft Excel** and analyzed using **SPSS version 22**. Qualitative variables were summarized using frequencies and percentages.

Ethical Considerations

Ethical clearance was obtained from the Institutional Ethics Committee (IEC Application No. 726 dated 04.06.2021). Written informed consent was obtained from all participants prior to inclusion in the study.

RESULTS

A total of **110 patients with clinically suspected subcutaneous mycoses** were assessed for eligibility during the study period. All patients meeting the inclusion criteria were included in the final analysis. No participants were excluded after recruitment.

Sociodemographic characteristics of the patients were given in table 1. Out of these 110 patients, 71.82% were male. The highest number of patients (71%) was in the age group of 20-40 years. Most cases occurred mainly in northern and western Odisha. The majority (68.18%) of patients were from low-income backgrounds, and 58.18



% of patients were residing in rural areas. Most of them were farmers (43.9%). Different clinical parameters of the patients were given in table2. In all, 33 % patients recalled having an injury prior to the development of infection, 18.19% patients were found to have involvement of bony

parts of the body due to infection. In our study, the most common site involved was the foot (38.18%), followed by the leg (27.27%). The duration of the disease ranged from 6 months to 3 years.

Table1: Sociodemographic characteristics of the patients(N=100)

Characteristics	Number(n)	Percentage (%)
Gender		
Male	79	71.82
Female	31	28.18
Age group		
1-10	00	00.00
11-20	08	7.27
21-30	31	28.18
31-40	48	43.63
41-50	14	12.72
51-60	09	8.20
Regions		
Northern	31	28.18
Southern	24	21.82
Western	33	30.00
Central	22	20.00
Socio-economic status		
Low	75	68.18
Middle	35	31.82
High	00	00.00
Rural vs urban		
RURAL	64	58.18
Semiurban	25	22.72
Urban	21	19.10
Occupation		
Farming	30	27.27
Gardener	23	20.90
Forest worker	17	15.45
Cattle caretaker	11	10.00
Housewife	09	08.20
Others	20	18.18

Prevalence of cases of subcutaneous mycoses: During the study period, a total of 110 clinically suspected cases were included. Diagnosis of subcutaneous mycoses by positive culture finding was 52.72 %, by direct microscopy (KOH mount) was 78.18%, and 80% cases were detected by histopathological finding. (Figure1)

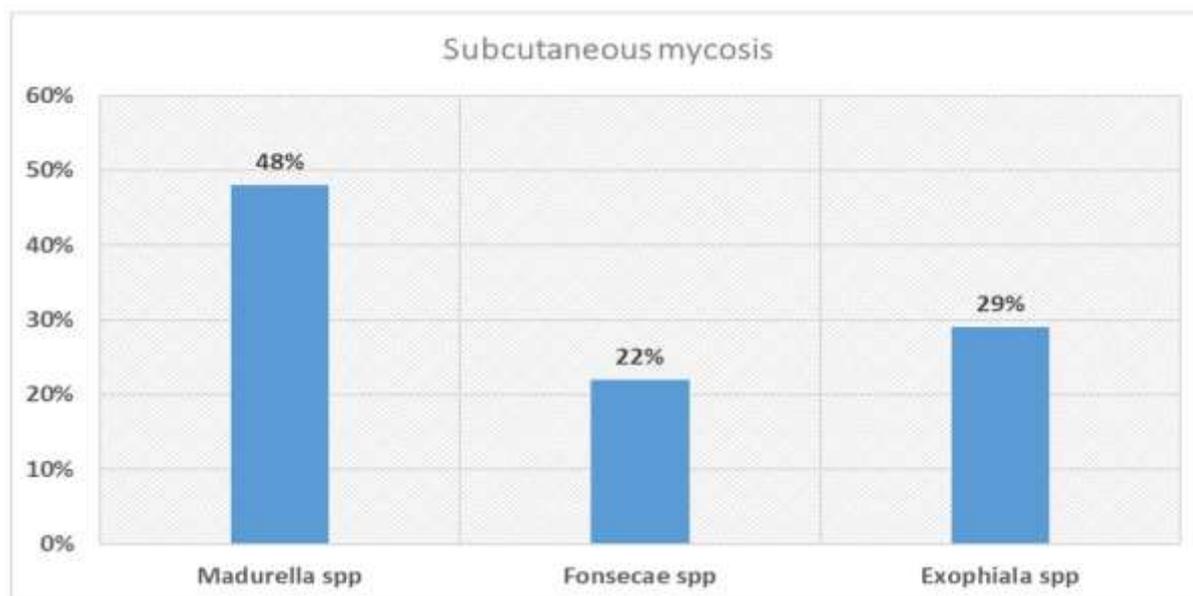


Figure 1: Distribution of fungal species isolated from culture-positive cases of subcutaneous mycoses

Table 2: Clinical parameters of the patients. (N=110)

Characteristics	Number(n)	Percentage (%)
History of trauma		
Present	33	30.00
Absent	77	70.00
Bony involvement		
Present	20	18.19
Absent	90	81.81
Site of involvement		
Foot	42	38.18
Hand	14	12.73
Leg	30	27.27
Arm	12	10.91
Abdomen	08	07.27
Thigh	02	01.82
Chest	01	00.91
Face	01	00.91
Duration of history		
<1yr	12	10.91
<2yr	43	39.09
<3yr	42	38.18
<4yr	13	11.82

Identification of various species causing subcutaneous mycoses:

Among the culture-positive mycosis cases, 28(48.2%) were Madurella spp, 17(29.31%) were Exophiala spp, and 13(22.41%) were Fonsecae spp. (Figure2)

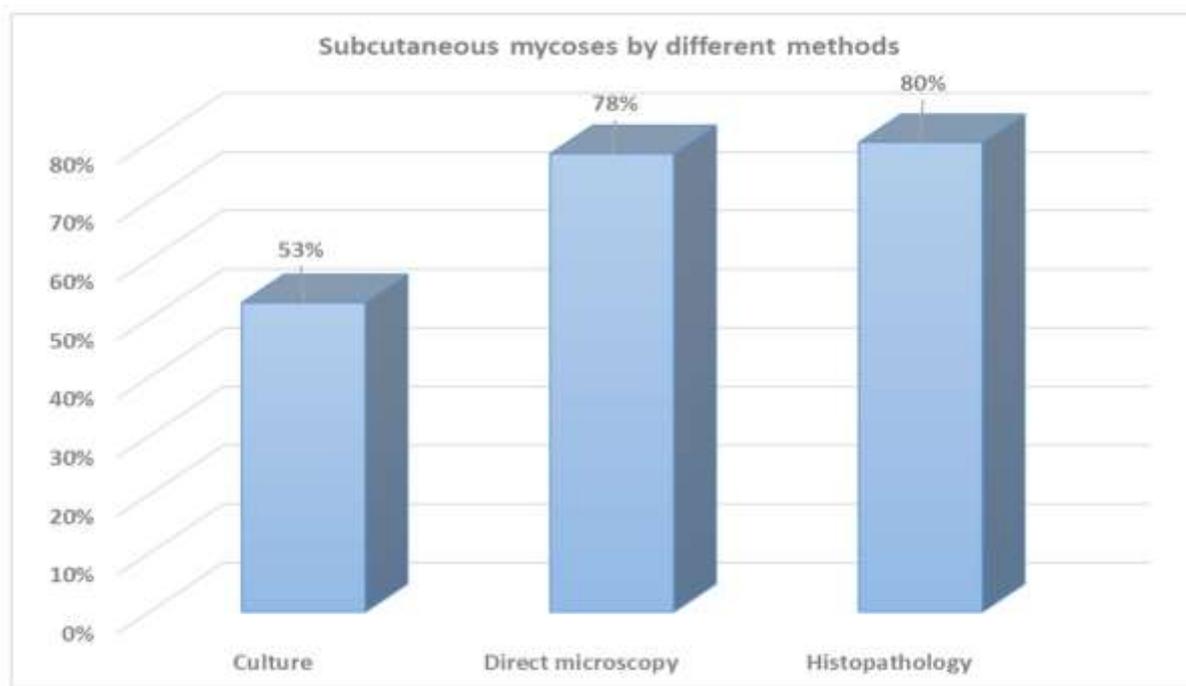


Figure 2: Diagnostic detection of subcutaneous mycoses by KOH mount, fungal culture, and histopathology.

Table 3: Diagnostic probability of KOH and Culture compared with Histopathology. (N=110)

Test		Histopathology	
		positive (88)	negative (22)
KOH	Positive (86)	84	02
	Negative (24)	04	20
Culture	Positive (58)	57	01
	Negative (52)	31	21

Diagnostic probability of Different tests: Diagnostic probability of KOH and Culture compared with Histopathology was given in Table 3. The sensitivity of KOH was 95%, and that of Culture was 65%. Specificity of KOH was calculated to be 91%, and that of culture was

95%. PPV and NPV of KOH were 77% and 83%, respectively. For culture, PPV and NPV were 98% and 40%, respectively. **Antifungal susceptibility test of the isolated fungus.** Antifungal susceptibility testing of the culture-positive cases is given in Table 4.

Table 4: Antifungal susceptibility testing (AFST) of the culture-positive cases (N=58)

AFST	AND	FLU	ITRA	POS	Amp-B
Resistant	58	58	2	5	7
Intermediate	-	-	-	-	-
Sensitive	-	-	56	53	51

All fungal isolates were resistant to Fluconazole (FLU) and Anidulafungin (AND). Most of the isolates were sensitive to Itraconazole (ITRA), Posaconazole (POS), and Amphotericin-B(Amp-B)

Discussion

Among the 110 patients, 72% were males and 28% were females, which was similar to the earlier studies by Ramesh M. Bhat et al. [9]. The frequency of fungal infections were more in males, which was due to the fact



that they were more exposed to an environment conducive to the spread of organisms. The most common age group affected was 20-40 years (71%), which is similar to a study by Ramesh M. Bhat et al [9], but the youngest age was 14 years, and the mean age of patients with subcutaneous infection was 49.4 years but in this study, the mean age was 33 years. The differences can be explained by the fact that there were no children or no adults above 60 yr. age reported in this study. It was in contradiction to the study of S.J Hashemi et al., who found that the 41-50 year age group was the most affected.[16] In most cases, 58% were reported from northern and western Odisha. Earlier cases were reported in Panigrahi A et al., and Sarangi G et al. explain that such a higher incidence is due to the geographical and socioeconomic situation. Northern and western Odisha are mainly hilly areas. Due to low socio-economic status, they are mostly unaware of taking proper care of themselves while working in fields. [17,18] In our study, we observed that 75 cases were from low socio-economic status and 35 were from middle socio-economic status. The study by Ramesh M. Bhat et al also showed the same findings. [9] In this study 58 % of patients were from rural areas and 22 % were from semi urban areas, similarly study by Ramesh M. Bhat et al also showed that subcutaneous mycoses were higher in rural areas[9] This could be because, most of the times subcutaneous mycoses are occurring due to inoculation of organism after a trivial trauma which is most often not known. In this study, the most common occupation associated with subcutaneous mycoses was farmers and similar work groups (62%). The study by Ramesh M. Bhat et al also showed agriculture as the main occupation, especially among rubber tappers. [9] Occupational exposure represented an important risk factor for subcutaneous mycoses; this may be related to trauma insensitivity when engaged in agricultural activities.

A history of trauma was seen in 30% of patients in this study. There is a history of injury prior to the development of lesions, as per the results of Queiroz-Telles et al.'s study, which found a history of trauma in 45% chromoblastomycosis cases. [19] Twenty cases out of 110 (18%) were found to have involvement of bony parts of the body due to infections caused by fungi, causing subcutaneous mycoses, which is a different finding than previous studies. The lower extremities were the most common site affected in 66 % of the subcutaneous fungal infections in this study. Similarly, a study by Ramesh M. Bhat et al also showed lower limb involvement in 64% of the study population. [9] In our study, the duration of disease ranged from 6 months to 3 years. Mean duration was 2.53 years. In contrast to the study by Ramesh M. Bhat et al., in which the duration of illness was about 4 months to 20 years. [9]

In fungal culture, a total of 58 (52.72%) of the samples showed growth. A total of 88(80%) samples were positive on histopathology examination, whereas no of positive findings in KOH mount was 86 (78.18%). In fungal culture, a total of 58 (52.72%) of the samples showed growth. This study has similar findings to the study of Sarangi G et al. [17]. Mostly *Madurella* spp (28 no) were isolated, followed by *Exophiala* spp (17 no), then *Fonsecaea* spp (13 no). This is in contrast to a study by Ramesh M. Bhat et al., in which chromoblastomycosis was the commonest subcutaneous fungal infection seen in 64% patients, followed by actinomycotic mycetoma in 16%. [9]

In the AFST test, the positive cultures were tested against amphotericin B, itraconazole, fluconazole, posaconazole, and anidulafungin. All samples were resistant to fluconazole and anidulafungin. But most of the samples were sensitive to itraconazole, posaconazole, and amphotericin B. Itraconazole is the most common choice of first-line agents [20]. Itraconazole and terbinafine are presently the major antifungal agents considered for the medical management of subcutaneous mycosis, as per the study of Bonifaz A et al. [21]

Generalizability

The findings of this study are particularly relevant to tropical and rural settings where occupational exposure to soil and vegetation is common. Similar epidemiological patterns may be observed in other regions with comparable environmental and socio-economic conditions.

Limitations

This study has several limitations. It was conducted at a single tertiary care center, which may limit the generalizability of the findings to other geographic regions. The sample size was relatively small, and molecular diagnostic techniques were not used for fungal identification. Future multicenter studies with larger sample sizes and advanced diagnostic methods are recommended.

Conclusion

Prevalence of subcutaneous mycoses by positive culture finding was 52.72%, by direct microscopy (KOH mount) was 78.18%, and by histopathological finding was 80%. The majority of the fungal isolates were *Madurella* spp(48.27%), *Exophiala* spp (29.31%), and *Fonsecaea* spp (22.41%). All fungal isolates were resistant to Fluconazole (FLU) and Anidulafungin (AND). Most of the isolates were sensitive to Itraconazole (ITRA), Posaconazole (POS), and Amphotericin-B(Amp-B).



Recommendation

Increased awareness and early diagnosis of subcutaneous mycoses are crucial, particularly for members of rural and low socioeconomic communities involved in agricultural activities, according to the study's findings. To lower the danger of traumatic inoculation of fungal pathogens, farmers and field workers should be made aware of the significance of wearing gloves, protective gear, and shoes. To increase awareness of early symptoms and the necessity for timely medical consultation, health education activities in rural and mountainous areas of northern and western Odisha should be reinforced. To stop disease progression and consequences such as bone involvement, screening and early laboratory detection utilizing KOH mount, histopathology, and fungal culture should be promoted in suspicious cases. To determine the best course of treatment, antifungal susceptibility testing should be taken into consideration wherever feasible. While fluconazole and anidulafungin may be avoided due to documented resistance, itraconazole, posaconazole, and amphotericin B should be regarded as appropriate treatment choices because the majority of isolates demonstrated sensitivity to these drugs.

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List of Abbreviations:

FLU	Fluconazole
AND	Anidulafungin
ITRA	Itraconazole
POS	Posaconazole
Amp-B	Amphotericin-B
IEC	Institutional Ethics Committee
SDA	Sabouraud Dextrose Agar

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

SK: Data collection, laboratory work, manuscript drafting
MS: Data analysis and manuscript editing
SKh: Study design, supervision, and final approval of the manuscript

Author Biography

Dr. Sajid Khan is a Senior Resident in the Department of Microbiology at Fakir Mohan Medical College, Odisha,

with research interests in medical mycology and infectious diseases.

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