



Original Article

Phenotypic Identification of Fungal Isolates in Otomycosis: Role of Conventional Laboratory Methods in a Tertiary Care Setting

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ABSTRACT

Background: *Otomycosis* is a common fungal infection of the external ear, particularly in tropical regions. It often resembles bacterial otitis externa, making diagnosis challenging, and relies on basic laboratory methods in resource-limited settings. To study the importance of conventional phenotypic laboratory methods in identification of fungal isolates in *otomycosis*. The aim was to look for fungal elements by direct microscopy (KOH mount); for any isolate using morphological method (Gram stain and LPCB) and species level identification (germ tube test and CHROMagar).

Materials & Methods: A total of 97 clinically suspected cases of otomycosis were included in this laboratory-based cross-sectional study conducted at a tertiary care hospital. Samples were evaluated microscopically via KOH mount and cultured in a Sabouraud Dextrose Agar. We used Gram staining, *Lactophenol* Cotton Blue mount, germ tube test and *CHROMagar* for identification of fungal isolates. Statistical analysis was performed on data.

Results: Fungal growth was observed in 62.89% of cases, mainly *Candida albicans* (23.71%) and *Aspergillus niger* (16.49%). KOH detected fungi in 54.60% cases, while culture showed higher detection, including 14.43% cases where KOH was negative but culture was positive. Microscopy differentiated fungal types—Gram staining identified *Candida* (50.82%) and LPCB mount identified *Aspergillus* (49.18%). *Candida* species were confirmed by germ tube test, with 74.19% as *albicans*, and species-level identification was further achieved using *CHROMagar*.

Conclusion: Conventional methods like microscopy, culture, and biochemical tests remain essential for identifying fungal pathogens in *otomycosis*. A stepwise approach improves detection and supports effective treatment, especially in resource-limited settings.

Keywords: *Otomycosis*, *Fungal identification*, *KOH mount*, *CHROMagar*, *Germ tube test*, *Aspergillus*.

INTRODUCTION

Otomycosis is a fungal infection occurring in the external auditory canal which is commonly seen in otolaryngology practice especially in tropical and subtropical areas [1-3]. Symptoms including pruritus, *otalgia*, aural fullness, *otorrhea*, and hearing impairment often overlap with bacterial otitis externa, thereby posing a challenge to clinical diagnosis [2, 5]. The condition develops when the natural defense mechanisms of the external auditory canal, including cerumen and its acidic pH, are disrupted, allowing fungal spores to colonize and proliferate [6, 7]. *Otomycosis* is not life-threatening but it recurs often and needs correct diagnosis for management [8]

Fungi, particularly *Aspergillus* and *Candida* species, have been implicated in otomycosis, with the most common fungi being *A. niger* and *C. tropicalis* [9-11]. Of these, *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* are the most frequently reported; however, species distribution varies with geographic and environmental factors [12-14]. The appearance of non-*albicans* *Candida* species and sporadic mixed infections adds to the mycological panorama [15]. Accurate identification of fungal isolates is crucial for diagnosis and management of otomycosis [16].

While suggestive clinical features are present, a clinical examination cannot reliably diagnose otomycosis due to overlap with findings from bacterial infections [2, 17]. The direct microscopic examination of KOH mount is a rapid and cost-effective screening test of fungal elements, however, it may fail to detect all cases [18]. Fungal culture remains the gold standard for laboratory confirmation and species-level identification of fungal pathogens [19]. Also, there are important conventional phenotypic methods such as Gram staining, LPCB (Lactophenol Cotton Blue) mount, germ tube test, and chromogenic media that help in differentiating fungal species on the basis of morphological characters [20].

In such circumstances, conventional laboratory techniques remain reliable and practical approaches for the identification of fungal pathogens. A combined stepwise approach involving microscopy, culture, and phenotypic identification facilitates the diagnosis and classification of fungal isolates. Therefore, the present study aims to evaluate the role of conventional phenotypic laboratory methods in identifying fungal isolates in otomycosis in a tertiary care setting, by detecting fungal elements using KOH mount, identifying isolates through morphological methods (Gram stain, LPCB) in conjunction with culture findings, and performing species-level identification using tests like germ tube and CHROMagar to assess their diagnostic utility.

MATERIALS AND METHODS

This laboratory-based analytical cross-sectional study was conducted in the Mycology Laboratory, Department of Microbiology, Uttar Pradesh University of Medical Sciences (UPUMS), Saifai, Etawah, Uttar Pradesh, India, from April 2024 to December 2025.

The study was approved by the Institutional Ethics Committee (Approval No. 24/2024-25). A total of 97 ear swab specimens were collected from patients with suspected otomycosis and processed for direct microscopy, fungal culture, and phenotypic identification of fungal isolates.

Isolation: The first specimen was examined by 10–20% potassium hydroxide (KOH) mount for the detection of fungal elements. The second specimen was inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 25°C and 37°C for fungal culture. The culture plates were examined periodically for fungal growth. Fungal isolates were processed for identification using Gram staining, Lactophenol Cotton Blue (LPCB) mount, germ tube test, CHROMagar, and other conventional phenotypic methods, wherever applicable.

Identification: Fungal isolates were identified based on their cultural and microscopic characteristics. *Candida* isolates were further differentiated on CHROMagar according to colony colour and morphology. Light green colonies were suggestive of *Candida albicans*, whereas other *Candida* species produced distinct characteristic colours. Final species identification was confirmed using conventional phenotypic methods, including microscopic examination and relevant biochemical tests

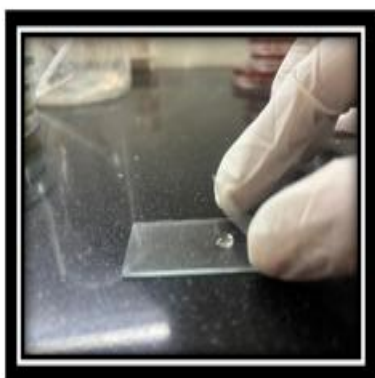


Fig 1(i): Slide KOH Method

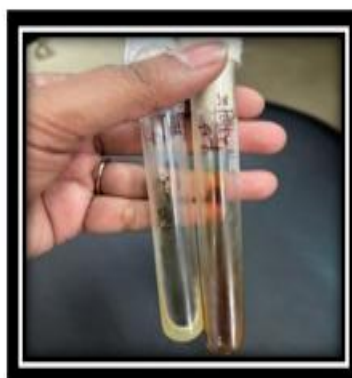


Fig 1(ii) & (iii): *Aspergillus niger* on SDA



Fig 1(iv): Germ tube test positive

Statistical Analysis: Statistical analysis of data was conducted using SPSS version 27. It used descriptive statistics and results were expressed in frequencies and percentages.

RESULT

In the present study, we aimed to determine the prevalence of otomycosis among clinically suspected cases and analyse their demographic distribution. Out of a total of 97 clinically suspected patients, 61 (62.9%) were females and 36 (37.1%) were males. The overall mean age was 35.1 years among males and 33.7 years among females. Fungal growth was confirmed in 61 patients, resulting in an overall otomycosis prevalence of 62.8% in the study population.

Table 1: Culture Outcomes and Distribution of Fungal Isolates in Otomycosis Patients

Fungal Isolates	Species	Number of Patients	Percent (%)
<i>Aspergillus</i> species	<i>Aspergillus niger</i>	16	16.49%
	<i>Aspergillus flavus</i>	13	13.40%
	<i>Aspergillus terreus</i>	1	1.03%
<i>Candida</i> species	<i>Candida albicans</i>	23	23.71%
	<i>Candida dubliniensis</i>	1	1.03%
	<i>Candida krusei</i>	2	2.06%
	<i>Candida tropicalis</i>	5	5.15%
No fungal growth / Contamination		36	37.11%
Total		97	100.00%

Table 2: Direct KOH Examination Findings in Otomycosis Patients (n = 97)

Direct KOH Findings	Number of Patients	Percent (%)
Fungal element seen	53	54.60%
No fungal element seen	44	45.40%

Table 3: Comparison of KOH Examination and Culture Results (n = 97)

Test Result Category	Number of Patients	Percent (%)
Total KOH Positive	53	54.64%
Total Culture Positive	61	62.89%
Both KOH & Culture Positive	47	48.45%
KOH Negative & Culture Positive	14	14.43%
Contamination	36	37.11%

Table 4: Distribution of Microscopic Findings in Otomycosis Patients (n = 61)

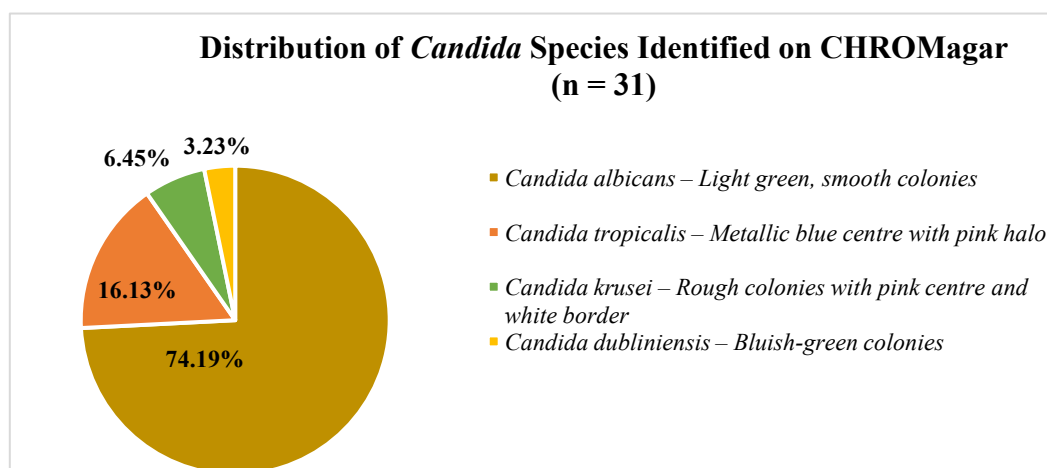
Fungal Organism	Microscopic Method	Characteristic Findings	Number of Patients	Percent (%)
<i>Aspergillus</i> species	Lactophenol Cotton Blue (LPCB) mount	Septate hyphae (long, branched hyphae suggestive of <i>Aspergillus</i> species)	30	49.18%
<i>Candida</i> species	Gram staining	Budding yeast seen / Pseudo hyphae (suggestive of <i>Candida</i> species)	31	50.82%

Table 5: Germ Tube Test Results for Identification of Candida Species (n = 31)

Germ Tube Test Result	Number of Patients	Percent (%)
<i>Candida albicans</i>	23	74.19%
<i>Candida non-albicans</i>	8	25.81%
Total	31	100%

Table 6: Distribution of Candida Species Identified on CHROMagar (n = 31)

CHROMagar Colony Characteristics	Number of Patients	Percent (%)
<i>Candida albicans</i> – Light green, smooth colonies	23	74.19%
<i>Candida tropicalis</i> – Metallic blue centre with pink halo	5	16.13%
<i>Candida krusei</i> – Rough colonies with pink centre and white border	2	6.45%
<i>Candida dubliniensis</i> – Bluish-green colonies	1	3.23%
Total	31	100 %

**Fig 4: Distribution of *Candida* Species Identified on CHROMagar (n = 31)**

DISCUSSION

This study evaluated the effectiveness of conventional phenotypic laboratory methods used to identify fungal isolates in otomycosis. According to data presented in Table 1, fungal growth obtained in 61 out of 97 cases. In this *Candida* and *Aspergillus* species contributed almost equally to the etiological profile. The most common isolates were *Candida albicans* (23.71%) and *Aspergillus niger* (16.49%). The studies of Prasad et al. and Pontes et al. reveal similar findings [11, 21]. Furthermore, Barati et al. and Bojanović M et al. noted *Aspergillus* predominance [13,22]. This indicates that local/regional geographical and/or environmental factors matter.

As shown in Table 2, the fungal elements were detected in 54.60 percent of the cases by KOH mount indicating moderate sensitivity, a significant number remaining undetected. The direct microscopy limitations were also reported by Kulal et al. and Howlader et al. [18,23]. An indication of KOH mount is not sufficient for confirmation diagnosis in all cases.

According to a further comparison in table 3, positivity by culture (62.89%) is higher than positivity by KOH (54.64%). Out of the KOH-negative, culture-positive cases were 14.43 %. As per the findings of Aneja et al. and Rawat et al. [19, 24], fungal culture detects additional cases missed by microscopy, and is the gold standard when used in combination with direct examination.

The results presented in Tables 4 to 6 further confirmed the utility of the phenotypic identification methods. Gram staining and Lactophenol Cotton Blue (LPCB) mount effectively differentiated between *Candida* (50.82%) and *Aspergillus* (49.18%) species, which are in accordance with Larone and Jeer et al. [20, 25]. Germ tube testing of the isolates in Table 5 shows that *candida albicans* formed 74.19% of the total isolates. *Candida albicans* was confirmed by Swetha et al. and Viswanatha et al. [15, 26]. Furthermore, Table 6 shows CHROMagar provided clear identification of species *C*. Most common is *albicans* followed by *c tropicalis*, *C. C. krusei* and *C. dubliniensis*. The findings were similar to that of Rawat et al. and Ahmed et al. [24, 27]. The results support a stepwise diagnosis using microscopy, culture, and phenotype to achieve accurate and reliable identification of otomycosis.

CONCLUSION

The current study emphasizes that otomycosis is a common fungal infection that cannot be accurately diagnosed based on clinical findings alone. Culture-proven fungal growth was observed in a large number of cases, with *Candida* and *Aspergillus* species being most common.

The use of the KOH mount, culture method, Gram stain, LCB mount, germ tube test as well as CHROMagar proved to be useful for detection and identification of fungal isolates at species level. Although KOH mount is a useful preliminary screening test, its fungal culture detection rate was higher and an essential confirmatory test.

According to the authors, details a stepwise diagnostic approach using microscopy, culture, and phenotypic identification methods for identification of fungal infection in otomycosis. In business settings as well as resource-poor countries, such means can help clinicians make timely decisions and manage appropriately.

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