



Identification of Fungal Pathogens in the Patients with Otomycosis: A One-Year Observational Study at a Tertiary Care Hospital in Dhaka City of Bangladesh

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Abstract

Background: Accurate diagnosis of otomycosis depends on reliable microscopy and culture, supported by adjunctive tools such as PCR. **Objective:** This study was carried out to isolate and identify Fungal pathogens both phenotypically and genotypically among the patients with otomycosis. Phenotypic methods offer cost-effective routine diagnosis with PCR aiding timely and targeted therapy. **Methodology:** The clinically suspected patients of otomycosis attending in the OPD of otolaryngology department of Dhaka Medical College Hospital, Dhaka were studied by Cross sectional observational study from January 2024 to December 2024. All aural swab samples were processed for direct microscopic examination and culture. Species identification was performed using colony morphology, microscopic features and biochemical tests with polymerase chain reaction employed for confirmation of Fungal pathogens. Species identification guides targeted antifungal therapy and reduces complications. **Results:** Out of 386 patients of otomycosis *Candida* species 51 (13.21%) was the most common isolated fungal pathogen followed by *Aspergillus species* 29 (7.51%) and no growth of organism was seen in 306 (79.27%) samples. **Conclusion:** The present study highlights that *Candida albicans* and *Aspergillus niger* were the most prevalent fungal isolates among all clinically suspected patients of otomycosis in a tertiary care hospital in Dhaka.

Keywords: Causative agents, Etiology, Otomycosis, *Candida albicans*, *Aspergillus niger*, Prevalence.

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Introduction

Otomycosis is a frequently encountered fungal infection that involves the external auditory canal (EAC). It can manifest in acute, subacute, or chronic forms and is most often unilateral, bilateral involvement is more frequently observed in immunocompromised individuals. Clinically, it is marked by inflammation, pruritus and scaling. This condition occurs worldwide, with reported prevalence rates ranging from 9.0% to 30.0% among patients

exhibiting symptoms of EAC infection. The primary etiological agents are molds from the *Aspergillus* genus and yeasts from the *Candida* genus, notably *Aspergillus niger* and *Candida albicans*. The incidence of otomycosis is influenced by socioeconomic factors and geographic location, with higher prevalence noted in tropical country like Bangladesh with warm and humid climate¹.

Immunocompetent hosts may also be affected by this fungal infection, which can present with complications such as hearing loss and invasive infection of the temporal bone. Patients with diabetes or lymphoma, as well as those undergoing or receiving chemotherapy or radiation therapy, are at increased risk of developing potential complications of otomycosis².

The incidence of tympanic membrane perforation in otomycosis found to be 11.0% in Asia. The

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pathophysiology of this complication is avascular necrosis of the tympanic membrane (TM) as a result of mycotic thrombosis in the adjacent blood vessels³. Various host factors (local, systemic) and environmental factors can predispose a person to otomycosis. Proper identification of causative agents is mandatory in order to prevent recurrences and complications⁴. Fungi are classified as molds, yeasts, or a combination of both forms⁵. Molds reproduce by forming spore, one of the most famous of them are *Aspergillus* species⁶. Among fungi identified as human pathogens, *Candida* species are frequently isolated from human fungal infections⁷.

Otomycosis, presents a difficult and distressing condition for patients as it frequently requires long-term treatment and follow up. Despite this, there could be recurrence⁸. Other *Candida* species such as *Candida parapsilosis*, *Candida guilliermondii*, *Candida tropicalis* and *Candida glabrata* have also been isolated and identified as etiological agents of the external auditory canal infection, although with relatively low incidence⁹. Among *Aspergillus* species, *Aspergillus niger* is usually the predominant species, followed by *Aspergillus flavus* and *Aspergillus fumigatus*¹⁰. *Aspergillus* species are associated with several health problems¹¹.

Currently used methods for the isolation and identification of *Candida* species are the culture, germ tube test, CHO assimilation test, sugar fermentation test, chromogenic culture media, VITEK and molecular techniques¹². Culture in Sabouraud's Dextrose Agar (SDA) media takes 24 to 48 hours to grow. The germ tube test is a standard test used in laboratories for the identification of *Candida albicans*, because it is cheap, quick and simple. Sugar fermentation test has been conventionally used in *Candida* species identification due to relative simplicity, low cost and ready availability of standardize protocols. Chromogen based culture medium has shown a high degree of accuracy, it also can be used for detection and identification of *Candida* species directly from clinical specimens¹³. The PCR for detection and identification of *Candida* species is simple, rapid, sensitive and highly reproducible¹⁴.

Accurate diagnosis relies on reliable microscopy and culture, aided by adjunctive tools such as PCR. The treatment of otomycosis is becoming difficult day by day as antifungal resistance is on rise. This study was done to isolate and identify Fungal pathogens by conventional methods and polymerase chain reaction.

Methodology

Study Settings and Population: This cross-sectional observational study was carried out in the Department of Microbiology at Dhaka Medical College, Dhaka, Bangladesh from January 2024 to December 2024 for a period of one year. All the clinically suspected patients of otomycosis irrespective of age and sex from OPD of Otolaryngology department of Dhaka Medical College Hospital, Dhaka were selected for this study. The patients with chronic suppurative otitis media, history of active otitis media, tympanic membrane perforation, prior ear surgery or aural procedure, patients who received treatment with antifungal drugs for last two weeks, patients or legal guardian who did not give informed written consent to participate were excluded from this study.

Study Procedure: A questionnaire was used for each case. All the relevant information and data were systematically recorded in a pre-designed data collection sheet. The aural swab samples were collected as per the standard recommended protocol and inoculation in Sabouraud's dextrose agar (SDA) media incorporated with chloramphenicol and gentamycin and in Blood agar media supplemented with 5% sheep blood was done for primary isolation of fungi. Then a thin smear was made for wet mount preparation with 10% KOH, Gram's staining and microscopy was done. To differentiate between different species of *Candida* the *Candida* differential media (chromogenic agar), Germ tube test and VITEK 2 system were used. In case of *Aspergillus* species the microscopic characteristics of colony from primary culture was observed by lactophenol cotton blue staining. Subculture in Potato dextrose agar (PDA) media was done to observe mycelium and sporulation. Isolation and identification of fungi were done by colony morphology, staining and biochemical tests. Among biochemical tests, sugar fermentation test was done to identify different species of *Candida* and urease activity was carried out in Christensen's urea agar media.

Procedure of Conventional PCR: Major steps of PCR include DNA extraction, amplification in a thermal cycler, and analysis of the result. Fungal DNA extraction is done according to the manufacturer's instruction supplied with the Qiagen DNA extraction kit. ATCC strain of *Candida* species was collected and considered as control strain.

Primers used for PCR: The fungus positive samples were subjected for run with genus/species specific primers for molecular diagnosis.

Primers list for PCR: Primers used in this study for identification of *Candida albicans*¹⁵

Primers	Sequence of primers (5'-3')	Size of Product(bp)
ITS-1	F: GATACCGTCGTAGTCTTA	450-550 bp
ITS-4	R: ATTCCTCGTTGAAGAGC	450-550 bp

Primers used for identification of *Candida tropicalis* and *Candida parapsilosis*:¹⁶

Primers	Sequence of primers (5'-3')	Size of Product(bp)
<i>C. tropicalis</i>	F: CAATCCTACCGCCAGAGTTAT	500-583bp
CRT 1/2	R: TGGCCACTAGCAAAATAAGCGT	500-583bp
<i>C. parapsilosis</i>	F: GCCAGAGATTAACTCAACCAA	290-550bp
CPA 1/2	R: CTATCCATTAGTTTATACTCCGC	290-550bp

The primers sequence of 16S rRNA gene of *A. niger* and their product size.¹⁷

Primers	Sequence of primers (5'-3')	Size of Product(bp)
<i>F. A. nig</i>	GATTCGACAGCATTT(CT/TC)CAGAA	300-500bp
<i>R. A. nig</i>	AAAGTCAATCACAATCCAGCCC	300-500bp

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Categorical data were summarized in terms of frequency counts and percentages. In analysis of results, the data obtained were entered in Microsoft Excel sheet, Chi-square was used to determine the association between two categorical variables. Values of $p \leq 0.05$ were considered statistically significant.

Ethical issue: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the Research Review Committee (RRC) of the Microbiology department of Dhaka Medical College (DMC), Dhaka and the Ethical Review Committee (ERC) of DMC (ERC-DMC/ECC/2024/140). Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

This cross-sectional study was conducted to identify Fungal pathogens from otomycosis patients by conventional and molecular methods. A total of 386 samples were collected in present study from clinically suspected patients of otomycosis attending the outpatient department of otolaryngology department of Dhaka Medical College Hospital during the study period of January 2024 to December 2024 among which diagnosed cases were 80. The number of isolated fungal pathogens in clinically suspected otomycosis cases were measured (n=386). Isolated *Candida* species were 51 in number, isolated *Aspergillus* species were 29 in number and no growth of organism was seen in 306 cases of otomycosis (Table 1).

Table 1: Isolation of Fungal Pathogens in Clinically Suspected Cases of Otomycosis (n=386)

Isolated Fungal Pathogens	Number of Isolates	P Value
<i>Candida species</i>	51(13.21%)	0.001
<i>Aspergillus species</i>	29(7.51%)	
No growth	306(79.27%)	
Total	386(100.0%)	

p value was calculated by Chi-square test

The distribution of *Candida albicans* and non-*albicans Candida* were recorded among isolated *Candida species* (n=51). Among 51 isolates 45 (88.23%) was *Candida albicans* and 06 (11.76%) was non-*albicans Candida species* (Figure I).

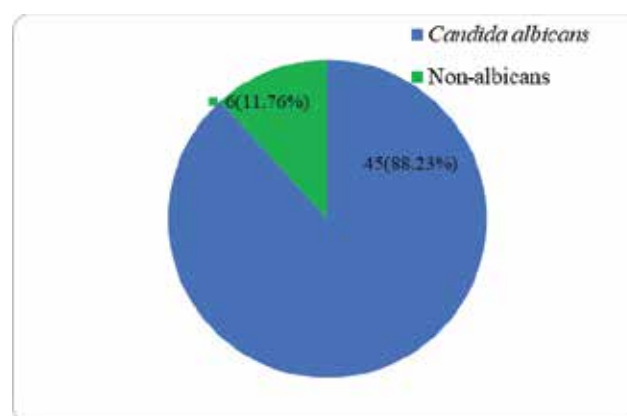


Figure I: Distribution of *Candida albicans* and non-*albicans Candida* among isolated *Candida species* (n=51)

The distribution of fungal pathogens was recorded in aural swabs (n=80). Out of 80 culture-positive isolates, *Candida albicans* constituted the majority 45 (56.25%), followed by *Candida tropicalis* 3 (3.8%), *Candida parapsilosis* 2 (2.5%), and *Candida ciferrii* 1

(1.25%). Among the *Aspergillus* species, only *Aspergillus niger* was isolated, comprising 29 (36.25%) of the total isolates (Table 2).

Table 2: Distribution of Fungal Pathogens in Aural Swabs (n=80)

Name of Fungi	Culture Positive
<i>Candida albicans</i>	45 (56.3%)
<i>Candida tropicalis</i>	3 (3.8%)
<i>Candida parapsilosis</i>	2 (2.5%)
<i>Candida ciferrii</i>	1 (1.3%)
<i>Aspergillus niger</i>	29 (36.3%)
Total	80(100.0%)

The distribution of fungus positive and negative cases were recorded by microscopy and culture among otomycosis patients (n=386). About 72 (18.7%) cases were positive by both microscopy and culture. Only microscopy missed 08 (2.1%) samples where culture missed 93 (24.1%) cases (Table 3).

Table 3: Distribution of fungus positive cases by Microscopy and Culture among Otomycosis patients (n=386)

Culture Result	Microscopy		Total
	Positive	Negative	
Positive	72(18.7%)	8(2.1%)	80(20.7%)
Negative	93(24.1%)	213(55.2%)	306(79.3%)
Total	165	221	386

Germ tube test was performed on 51 cases where 45 isolates of *Candida* were positive and remaining 6 of them were germ tube test negative. Among these 6 negative isolates 3 were identified as *C. tropicalis*, 2 as *C. parapsilosis* and 1 as *Candida ciferrii* by VITEK 2 system. One isolate remained unidentified by VITEK 2 system which was germ tube test positive (Table 4).

Table 4: Detection of Different *Candida* species by Germ tube test and VITEK 2 system

Name of <i>Candida</i> Species	Germ Tube Test		VITEK 2
	Positive	Negative	
<i>Candida albicans</i>	44	-	44
<i>Candida tropicalis</i>	-	3	3
<i>Candida parapsilosis</i>	-	2	2
<i>Candida ciferrii</i>	-	1	1
Unidentified	1	-	-
Total	45	06	50

The identification of *Candida species* was performed by chromogenic agar media and VITEK 2 compact system. Among 45 isolates of *Candida albicans* 37 (72.5%) were identified by CHROM agar and 44 (86.27%) by VITEK 2 system. Among 3 isolates of *Candida tropicalis* 1 (1.96%) by CHROM agar and 3

(5.8%) by VITEK 2 system were identified. Among 2 isolates of *Candida parapsilosis* 1 (1.96%) by CHROM agar and 2 (3.9%) by VITEK 2 system and among 1 isolate of *Candida ciferrii* 0 (0.0%) by CHROM agar and 1 (1.96%) by VITEK 2 system were identified. One isolate remained unidentified by both CHROM agar and VITEK 2 system which was germ tube test positive (Table 5).

Table 5: Identification of *Candida* species by Chromogenic Agar Media and VITEK 2 Compact System

Species identified	Identified by Chromogenic Media	Identified by VITEK 2 System
<i>Candida albicans</i> (n=45)	37 (72.5%)	44 (86.27%)
<i>Candida tropicalis</i> (n=3)	1 (1.96%)	3 (5.8%)
<i>Candida parapsilosis</i> (n=2)	1 (1.96%)	2 (3.9%)
<i>Candida ciferri</i> (n=1)	0 (0.0%)	1 (1.96%)
Unidentified (n=1)	0 (0.0%)	0 (0.0%)
Total	39	50

The distribution of *Candida species* and *Aspergillus species* were identified by PCR among culture positive isolates. Out of 80 isolates, 51 (63.8%) were *Candida species* and 29 (36.2%) were *Aspergillus niger*. Among the 51 isolated *Candida species* 45 (56.25%) were *Candida albicans* and 6 (7.5%) were non *albicans Candida*. Among NACs, 3 (3.75%) were *C. tropicalis*, 2 (2.5%) were *C. parapsilosis* detected by PCR. One isolate of *Candida ciferrii* remained unidentified by PCR (Figure II).

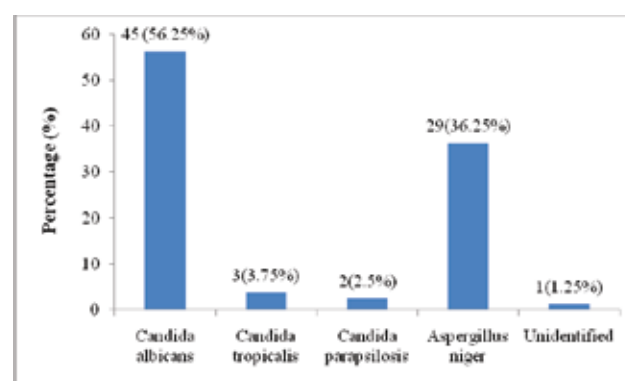


Figure II: Distribution of culture positive fungi identified by PCR (n=80)

The detection of *Candida* and *Aspergillus* species were analyzed by biochemical tests (Urease test and Sugar fermentation test) and PCR from culture positive isolates. Among 80 culture positive isolates 51 isolates of *Candida species* were only sugar fermentation test positive, while 05 isolates of *Aspergillus niger* were urease test positive. Among 80 culture positive isolates 79 were PCR positive (Table 6).

Table 6: Detection of *Candida* and *Aspergillus* species by biochemical tests (Urease test and Sugar fermentation test) and PCR from culture positive isolates (n=80)

Fungal pathogens	Biochemical Test		PCR
	Urease Test	Sugar Fermentation Test	
<i>Candida species</i> (n=51)	-	51	50
<i>Aspergillus species</i> (n=29)	05	-	29
Total (n=80)	05	51	79

Discussion

Otomycosis is a common superficial fungal infection of the external auditory canal present globally. The rate of otomycosis in this study was 20.7% (80 out of 386 cases). Our finding aligns with reports by Nipa et al¹⁹ and Kumar et al²⁰ with suspected case rates of 21.3% and 22.1%, respectively. These comparable prevalence rates indicate that otomycosis remains a common clinical issue, particularly in humid and tropical regions, where fungal infections are more due to favorable environmental conditions.

The use of multiple diagnostic approaches in otomycosis is justified because no single method can reliably detect and identify all fungal pathogens with complete accuracy. Direct microscopy provides rapid preliminary evidence of fungal elements, while culture allows isolation and viability assessment of the pathogen. Biochemical tests and automated systems such as VITEK 2 improve species-level identification, and PCR offers high sensitivity and specificity²¹.

Microscopy detected fungal elements in 165 (42.7%) cases, whereas culture confirmed fungal growth in 80 (20.7%) cases. Notably, microscopy missed 2.1% of culture-positive cases, while culture missed 24.1% of microscopy-positive cases. This finding is consistent with those of Kumar²⁰ who reported that microscopy failed to detect fungal elements in 3.0% of culture-positive cases of otomycosis. The possible reason for this discrepancy is, this method has limitations, such as lower sensitivity, an inability to distinguish the species of causative agents, and difficulty in differentiating contaminants from infectious agents¹⁰. Microscopy is more feasible for routine practice in otomycosis because it provides rapid, direct evidence of fungal elements, allowing early diagnosis and prompt initiation of treatment. It is simple, inexpensive, and requires minimal laboratory infrastructure. Microscopy can detect both viable and

non-viable fungi, which explains its higher detection rate. Culture is considered the gold standard method for diagnosing otomycosis because it confirms the presence of viable fungi and allows definitive species identification. Culture method has limitations because it is time-consuming and may yield false-negative results due to low fungal load, prior antifungal use, or fastidious growth requirements. Additionally, contamination and overgrowth by other fungus can hinder fungal isolation, reducing its sensitivity in routine practice²².

Among the 80 culture-positive cases, *Candida* species (63.7%) were predominant with *Candida albicans* (56.25%) being the most frequent causative organism. Results were reported by Pontes et al²³ who found that *Candida* species accounted for 60% of all culture-positive otomycosis cases in their study. Among the *Candida* species, *Candida albicans* was identified as the most frequent causative organism.

In this study, *Aspergillus niger* accounted for 36.25% of the isolates. These findings are consistent with recent studies reporting non-*albicans Candida* (39.7%) species and *Aspergillus niger* (30.9%) are emerging pathogens by Bojanovic et al¹⁰.

The VITEK 2 system offers rapid, automated, and standardized identification of fungal pathogens, reducing turnaround time compared to conventional methods. It provides reliable species-level identification of yeasts and molds with minimal hands-on effort. This study showed one germ tube-positive isolate that remained unidentified by the VITEK 2 system, suggesting a possible mixed growth of *Candida* species, as the biochemical patterns may overlap or become masked. VITEK 2 may also have limited library entries for emerging or rare species²⁴.

Arya and Naimshree²⁵ reported that CHROM agar correctly identified *Candida albicans* in 90.0% of isolates and *Candida tropicalis* in 85.0%, with strong correlation to VITEK 2 results. This contrasts with this study, where CHROM agar showed markedly lower identification rates for *Candida tropicalis* and *Candida parapsilosis* and one isolate remained unidentified by both methods.

A current study by Kucukkaya et al²⁶ focused on rare *Candida* species and found that CHROM agar, when supplemented with molecular methods, successfully identified *Candida ciferrii* in 67% of cases. This differs from the present study where *Candida ciferrii* was not identified by CHROM agar at all. This difference is explained by the limitation of CHROM

agar, as *Candida ciferrii* does not produce a distinctive or characteristic colony color on this medium. As a result, it cannot be reliably differentiated from other non-*albicans* *Candida* species using CHROM agar alone.

Among 80 culture-positive isolates, 51 isolates of *Candida* species were positive only by sugar fermentation test. This is consistent with the findings of Giri and Kindo²⁷ who reported that sugar fermentation and assimilation tests correctly identified 94.8% and 92.3% of *Candida* isolates respectively, highlighting their utility in differentiating species based on carbohydrate utilization patterns. The sugar fermentation test is advantageous because it is simple, inexpensive, and useful for preliminary identification of *Candida* species in routine laboratory settings, especially where advanced molecular methods are unavailable. However, its limitation lies in limited specificity and longer turnaround time, as closely related *Candida* species may show similar fermentation patterns, leading to possible misidentification.

Among 29 isolates of *Aspergillus* species only 5 isolates of *A. niger* were urease test positive. This finding aligns partially with the review by Zakaria et al²⁸ which reported that urease activity is not a consistent trait among *Aspergillus niger* isolates rather emphasized that while *Aspergillus flavus* and *Aspergillus fumigatus* are more commonly urease positive, some *Aspergillus niger* strains may exhibit urease activity depending on environmental conditions and strain specific enzymatic expression. The urease test is advantageous as a simple and rapid supplementary test that can support the identification of *Aspergillus niger*. The limitation of urease test is low sensitivity and poor specificity, since many *Aspergillus niger* isolates are urease-negative and urease positivity may also occur in other fungal species, making it unsuitable as a diagnostic test.

Among the 79 PCR confirmed otomycosis cases, *Candida albicans* was the predominant species, identified in 45 (56.25%) cases, while *Aspergillus niger* was detected in 29 cases (36.3%). These findings align with recent studies, including those by Bojanovic et al¹⁰ which reported *Candida albicans* as the most common causative agent (65.2%) followed by *Aspergillus niger* (34.8%). PCR provides rapid, highly sensitive, and specific identification of fungal pathogens, including species that are difficult to differentiate by conventional methods. The limitations

of PCR include higher cost, requirement for specialized equipment and expertise, and the inability to distinguish between viable and non-viable fungi.

There are few limitations of the study. Reconfirmation of *Candida* species by Restriction Fragment Length Polymorphism (RFLP) could not be done. Reference ATCC strain of *Aspergillus niger* was not available.

Conclusion

This study highlights the high prevalence of otomycosis in Bangladesh, with *Candida albicans* being the predominant species followed by *Aspergillus niger* in otomycosis patients emphasizing the need for early detection of these fungi. These findings support evidence-based treatment protocols and help address the growing challenge of antifungal resistance, ultimately improving clinical outcomes and preserving auditory functions. PCR could be an alternative for rapid and accurate diagnosis of fungal pathogens where it is available.

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Conflict of Interest

The authors declare that they have no conflict of interests.

Financial Disclosure

No funds were received for this study.

Authors' contributions

Sharmin Shanjana conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Sharmin Shanjana contributed to the analysis of the data and interpretation of the results. Sazzad Bin Shahid and Mahbuba Chowdhury were involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

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