ORIGINAL ARTICLE

Investigation of *ERG11* gene prevalence among azole-resistant *Candida* species isolated from otomycosis patients

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ABSTRACT

Background: *Azole resistance in* Candida *species has emerged as a significant clinical challenge worldwide, particularly in patients with otomycosis associated with recurrent or progressive yeast infections.* ***Objective:*** *This study aimed to evaluate the antifungal efficacy of azole drugs against* Candida *species isolated from otomycosis cases and to investigate the distribution of the* ERG11 *gene associated with resistance.* ***Methodology:*** *A total of 114 clinical samples were collected from patients diagnosed with otomycosis.* Candida *isolates were identified using morphological methods. Antifungal susceptibility testing was carried out using the VITEK2 Compact System. The presence of the* ERG11 *gene was analyzed to determine resistance-associated genetic patterns among the isolates.* ***Results:*** *Females were more affected (54.38%) than males (45.61%), with the highest infection rate observed in the 31-40 years age group (20.2%). The VITEK 2 system evaluated five antifungal agents (*fluconazole, voriconazole, flucytosine, amphotericin B, *and* caspofungin*) and demonstrated variable susceptibility profiles among the isolates. Molecular identification revealed that* Candida albicans *(36.8%) was the most prevalent species, followed by* Candida parapsilosis *(26.3%),* Candida glabrata *(14.0%), and* Candida tropicalis *(12.4%), while* Candida krusei *showed the lowest prevalence (10.5%). PCR amplification of the* ERG11 *gene demonstrated an overall resistance rate of 75.4% and a sensitivity rate of 24.6%. Among the studied species,* Candida albicans *exhibited the highest resistance rate (85.7%), followed by* Candida tropicalis *(78.6%),* Candida parapsilosis *(73.3%),* Candida glabrata *(62.5%), and* Candida krusei *(58.3%).* ***Conclusions:*** *The study revealed a high prevalence of ERG11-related azole resistance among Candida species isolated from patients with otomycosis.*

INTRODUCTION

Otomycosis is an inflammatory lesion resulting from the invasion or excessive proliferation of pathogenic fungi in the external auditory canal[1]. The range of fungi responsible for human infections and the diversity of clinical manifestations linked to these infections have expanded[2]. Epidemiologic trends exhibit significant changes with emerging novel multidrug-resistant pathogenic fungi and broadening geographic ranges[3]. Recently, the management of yeast infections has become more challenging due to increasing complexity in diagnosis and treatment[4]. Additionally, multidrug-resistant yeast has been identified in patients who have not undergone antifungal treatment, which is highly concerning[5]. The progress of antifungal drugs has been impeded, in part, by the close evolutionary relationship between fungi and humans, which limits the availability of fungal-specific targets for therapeutic advancement[6].

Resistance to antifungal drugs is distinguished into two types: primary (intrinsic) and secondary (acquired)[7]. Innate resistance is genetically encoded and linked to fungal species regardless of drug exposure. Acquired (secondary) resistance, which arises from exposure to a certain factor, is frequently an antifungal agent or its structural analogue[8]. Fungal resistance to a specific antifungal compound extends to its entire class. Therefore, the resistance to any type of antifungal drugs can significantly limit the patient's treatment options[9].

Currently, azole antifungal drugs are commonly employed for the treatment of otomycosis, as they exhibit satisfactory bioavailability and broad-spectrum activity. Antifungal azoles can prevent yeast from growing by interfering with the ergosterol synthetic pathway[10]. Azole drugs inhibit the enzyme 14-α-demethylase, which is encoded by the *ERG11* gene[11]. This inhibition disrupts the formation and integrity of the yeast cell membrane, leading to increased membrane permeability and inhibition of yeast growth or cell death[12]. Unfortunately, there is an increasing number of resistant Candida species with long-term azole exposure. The possible mechanisms are linked to the efflux pumps, overexpression, and mutations in the genes of target proteins[13], as well as alterations in ergosterol biosynthesis or drug targets[14]. As fungal

resistance keeps getting worse, there is a growing need for new antifungal treatment options[15].

As a result, the rise of antifungal resistance has necessitated careful monitoring of fungal susceptibility, creating new antifungal drugs, and the adoption of prudent use strategies to reduce resistance[16]. In addition, advances in diagnostic testing, such as molecular techniques, help to identify resistant yeast faster and more accurately, making treatment more targeted[17]. Also, modern treatment strategies have concentrated on targeting pathways that are considerably different between fungi and humans, such as the biosynthesis of cell walls and the metabolism of sterols, to reduce collateral damage[14]. This study aims to determine the antifungal susceptibility patterns of Candida species isolated from otomycosis patients and to detect the *ERG11* gene associated with azole resistance.

METHODOLOGY

Patients and samples collection

The study was conducted in the Otolaryngology Departments of Basrah Teaching Hospital and Al-Sadar Teaching Hospital and was performed over 12 months from January to December 2025, including 114 male and female patients aged over 5 years. Those patients presented with various symptoms, including aural pain, itching, and otorrhea. Demographic information, risk factors, and clinical outcomes of the patients with a clinical diagnosis were evaluated and analyzed.

Culture and microscopic examination

Sterile swab samples were collected from patients diagnosed with otomycosis and directly streaked onto labeled sabouraud dextrose agar (SDA) plates. The inoculated plates were incubated at 37 °C for 5-7 days[18]. Colonies showing smooth, creamy, pasty, and opaque characteristics on SDA[19]. For microscopic examination, cultured yeast colonies were collected and smeared onto clean glass slides. The smears were stained using lactophenol cotton blue solution and then examined under a light microscope[20].

Antifungal Susceptibility Testing (AST)

The Vitek 2 compact device, equipped by BioMérieux, was used for antifungal susceptibility testing (AST) for fluconazole, voriconazole, flucytosine, amphotericin B, *and* caspofungin. The test was carried out according to the manufacturer's instructions[21]. The AST of the isolates was interpreted as sensitive (S), intermediate (I), and resistant (R) according to the Clinical and Laboratory Standards Institute (CLSI, 2025)[22].

Molecular Identification

Genomic DNA was extracted from each yeast isolate using the Presto™ Mini gDNA Yeast Kit according to the manufacturer's instructions[23]. Agarose gel electrophoresis was performed as a quality control step to confirm successful DNA extraction. The internal transcribed spacer regions (*ITS1-5.8S-ITS2*) of ribosomal DNA were amplified by PCR using the Maxime PCR PreMix (i-Taq) and specific primers: ITS1-F (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4-R (5′-TCC TCC GCT TAT TGA TAT GC-3′)[24]. The primer sequences were compared with sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm for primer design and sequence confirmation[25]. The total PCR reaction volume was 25 µL, and the reaction components were prepared according to the method described by described by Moody *et al.*[26]. The PCR amplification products were sent to Macrogen in South Korea for Sanger sequencing. The obtained sequences were aligned online with reference sequences of related genes available in the NCBI database using BioEdit software [27].

Molecular detection of the *ERG11* gene

The *ERG11* gene was detected using conventional polymerase chain reaction (PCR). The PCR reaction mixture was prepared in a total volume of 25 µL, consisting of 12.5 µL of Master Mix, 1 µL of each forward and reverse primer, 3 µL of template DNA, and 7.5 µL of nuclease-free deionized water (Thermo Scientific, USA). The primer sequences used for amplification of the *ERG11* gene are presented in (Table 1).

Table 1: Primer sequence of *ERG 11* gene of *Candida* species

Species	Primers sequences (5'-3')	Product size (bp)	Ref.
C. albicans	*ERG11*-F 5'- GCAGCTTCATATGGTCAACAACC-3' *ERG11*-R 5'- TAACATTGGCAACCCCATGAG-3'	100bp	[28]
C. glabrata	*ERG11*-F 5'- AGCTGCTTACTCCCACTTGACC-3' *ERG11*-R 5'-AGCTTGTTGGGCATGGTCTCTC-3'	412 bp	[29]
C. krusei	*ERG11*-F 5'- ACTCCTGTTTTCGGTAAGGGCG-3' *ERG11*-R 5'-CACCGGCACGCTTTGTATTG-3'	421bp	[30]
C. *tropicalis*	*ERG11*-F 5'-ATCCCACAGGCTTATTTGAAA-3' *ERG11*-R 5'-GGTCTCTTTCCTTGGTTTTG -3'	614bp	[30]
C. parapsilosis	*ERG11*-F 5'-CGAGATAATCATCA ACGAACATTC-3' *ERG11*-R 5'-AAAGACCGCATTGACTACCGAT-3'	648bp	[31]

Statistical Analysis

The data were statistically characterized by frequencies and relative frequencies. A probability value p-value less than 0.05 was considered statistically significant. All statistical calculations were done using IBM SPSS Statistics version 23 for Microsoft Windows.

RESULTS

Clinical and Phenotypic diagnosis

The present study included 114 patients with otomycosis, aged between 7 and 75 years. According to sex distribution, 62 (54.4%) of the isolates were obtained from females, while 52 (45.6%) were obtained from males. The distribution of otomycosis cases among different age groups showed a heterogeneous pattern, with the highest prevalence observed in the 31-40 years age group (20.2%), followed by the 41-50 years group (18.4%) and the 51-60 years group (16.7%). Regarding clinical manifestations, pruritus was the most frequently reported symptom, accounting for 38.6% of cases, followed by otalgia (28.9%) and otorrhea (20.2%). Ear fullness was observed in 8.8% of patients, whereas tinnitus was the least common symptom, recorded in 3.5% of cases. The findings also indicated that the right ear was more frequently affected by otomycosis (51.8%) than the left ear (36.0%), while bilateral infection was observed in 14 cases (12.3%), as illustrated in (Table 2).

Table 2. Demographics and clinical distribution of patients

Category	Characteristics	No.	(%)
Sex	Men	52	45.6
	Women	62	54.4
Age (years)	7-10	4	3.5
	11-20	9	7.9
	21-30	13	11.4
	31-40	23	20.2
	41-50	21	18.4
	51-60	19	16.7
	61-70	16	14.0
	71-80	9	7.9
Site of infection	Right ear	٥٩	51.8
	Left ear	41	36.0
	Bilateral	14	12.3

Antifungal susceptibility testing

A susceptibility test was conducted using five antifungals: fluconazole, flucytosine, voriconazole, amphotericin B, and caspofungin, which showed different levels of antifungal resistance, as shown in (Tables 3, 4, 5, 6, and 7).

Table 3. Fluconazole antifungal susceptibility profile of *Candida* species

Candida species	No. of isolates	Sensitive (S)	Intermediate / SDD	Resistant (R)
C. albicans	42	17 (40.5%)	4 (9.5%)	21 (50.0%)
C. parapsilosis	30	14 (46.7%)	5 (16.7%)	11 (36.7%)
C. glabrata	16	7 (43.8%)	2 (12.5%)	7 (43.8%)
C. tropicalis	14	8 (57.1%)	1 (7.1%)	5 (35.7%)
C. krusei	12	7 (58.3%)	1 (8.3%)	4 (33.3%)
Total	114	53 (46.5%)	13 (11.4%)	48 (42.1%)

Table 4. Flucytosine antifungal susceptibility profile of *Candida* species

Candida species	No. of isolates	Sensitive (S)	Intermediate / SDD	Resistant (R)
C. albicans	42	22 (52.4%)	4 (9.5%)	16 (38.1%)
C. parapsilosis	30	19 (63.3%)	2 (6.7%)	9 (30.0%)
C. glabrata	16	10 (62.5%)	1 (6.3%)	5 (31.3%)
C. tropicalis	14	9 (64.3%)	1 (7.1%)	4 (28.6%)
C. krusei	12	8 (66.7%)	1 (8.3%)	3 (25.0%)
Total	114	68 (59.6%)	9 (7.9%)	37 (32.5%)

Table 5. Voriconazole antifungal susceptibility profile of *Candida* species

Candida species	No. of isolates	Sensitive (S)	Intermediate / SDD	Resistant (R)
C. albicans	42	33 (78.5%)	2 (4.7%)	7 (16.6%)
C. parapsilosis	30	25 (83.3%)	1 (3.3%)	4 (13.3%)
C. glabrata	16	12 (75.0%)	1 (6.2%)	3 (18.7%)
C. tropicalis	14	11 (78.5%)	1 (7.1%)	2 (14.2%)
C. krusei	12	8 (66.6%)	1 (8.3%)	3 (25.0%)
Total	114	89 (78.0%)	6 (5.2%)	19 (16.6%)

Table 6. Caspofungin (Echinocandin) antifungal susceptibility profile of *Candida* species

Candida species	No. of isolates	Sensitive (S)	Intermediate / SDD	Resistant (R)
C. albicans	42	26 (61.9%)	7 (16.7%)	9 (21.4%)
C. parapsilosis	30	24 (80.0%)	2 (6.7%)	4 (13.3%)
C. glabrata	16	11 (68.8%)	2 (12.5%)	3 (18.8%)
C. tropicalis	14	10 (71.4%)	2 (14.3%)	2 (14.3%)
C. krusei	12	9 (75.0%)	1 (8.3%)	2 (16.7%)
Total	114	80 (70.2%)	14 (12.3%)	20 (17.5%)

Table 7. Amphotericin B antifungal susceptibility profile of *Candida* species

Candida species	No. of isolates	Sensitive (S)	Intermediate / SDD	Resistant (R)
C. albicans	42	38 (90.5%)	1 (2.4%)	3 (7.1%)
C. parapsilosis	30	26 (86.7%)	1 (3.3%)	3 (10.0%)
C. glabrata	16	14 (87.5%)	1 (6.3%)	1 (6.3%)
C. tropicalis	14	11 (78.6%)	1 (7.1%)	2 (14.3%)
C. krusei	12	10 (83.3%)	1 (8.3%)	1 (8.3%)
Total	114	99 (86.8%)	5 (4.4%)	10 (8.8%)

Molecular Identification

Molecular analysis using the internal transcribed spacer (*ITS1*- 5.8S- *ITS2*) region sequencing demonstrated notable variations in the frequency of *Candida* species between patient isolates. Among the identified species, *Candida albicans* was the most prevalent, accounting for 42 isolates (36.8%), followed by *Candida parapsilosis* with 30 isolates (26.3%). *Candida glabrata* was detected in 16 isolates (14%), while *Candida tropicalis* accounted for 14 isolates (12.4%), and the least frequently identified species was *Candida krusei*, with 12 isolates (10.5%), as shown in (Figure 1).

Detection of the *ERG11* gene

The DNA analysis was performed on 114 isolates. The *ERG11* gene was detected in 86 of the isolates, as shown in (Table 8).

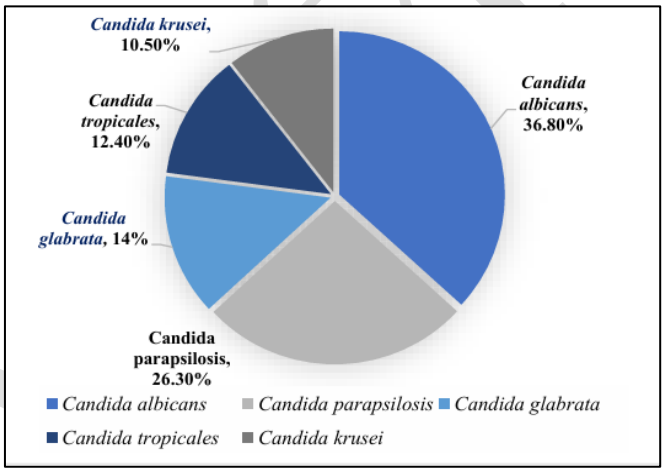

Fig. 1: Percentage distribution of *Candida* species identified by *ITS1-5.8S-ITS2 rDNA* sequencing among the yeast isolates

Table 8. Distribution of *ERG11*-associated azole resistance among *Candida* species

Candida species	No. of isolates	*ERG11* positive (resistant)	Resistance (%)	*ERG11* negative (sensitive)	Sensitive (%)
C. albicans	42	36	85.7	6	14.3
C. parapsilosis	30	22	73.3	8	26.7
C. glabrata	16	10	62.5	٦	37.5
C. tropicalis	14	11	78.6	3	21.4
C. krusei	12	7	58.3	5	41.7
Total	114	86	75.4	28	24.6

($\chi^2 = 5.88$, df = 4, P = 0.208)

DISCUSSION

Otomycosis is a prevalent clinical condition. Although not life-threatening, it can be an annoying condition for both the patient and the doctor because it requires long-term treatment, regular follow-ups, and the possibility of coming back. Azole antifungals are commonly employed in the treatment of Candida infections. Nonetheless, additional mechanisms in Candida species resistance to azoles are imperative for patient management[32]. This study observed a greater prevalence of otomycosis in females compared to males, consistent with the findings in previous research[33]. In contrast to certain studies indicating a higher prevalence of otomycosis in males[34], which reported 56% male cases and 44% female cases.

Otomycosis is typically diagnosed via clinical examination, with pruritus as the predominant symptom, succeeded by otalgia[35]. The most common symptoms of otomycosis in this study were itching (38.6%), ear pain (28.9%), and ear discharge (20.2%). 8.8% of patients experienced a feeling of fullness in the ear, which could have been caused by masses or fungal remnants blocking the ear. Tinnitus was the least reported symptom, affecting only 3.5% of patients. The mentioned complaints and their incidence were consistent with the findings of other studies[36]. However, some reports stated that otalgia was the most common. Otomycosis is usually a unilateral infection, infrequently involving both ears. In our study, the right ear was more often affected than the left ear (59.6% vs. 40.4%), which is similar to the study by Aremu et al.[35], only 12.7% of cases showed bilateral involvement, probably due to the fact that most people are right-handed and tend to clean their right ear more often with things that aren't sterile.

Automated systems, such as the VITEK ² system, have demonstrated their capacity to deliver swift and precise data regarding the susceptibility of *Candida* isolates, thereby aiding in prompt treatment decisions, when compared to traditional and alternative susceptibility testing method Weng et al.[37]. Demonstrated that all *Candida* species exhibited varying degrees of susceptibility to the antifungals employed. Studies by Bouchara et al.[38], indicated that yeasts are less sensitive to antifungals because the indiscriminate and frequent use of these drugs leads to the appearance of resistant *Candida* species. Consequently naturally, this *candida* differs from one species to another. Repeated use of antifungals causes mutations that make Candida more resistant and virulent. In the present study, Candida species were identified by PCR. *Candida albicans* was the most commonly observed species, followed by *Candida parapsilosis*. Our findings are consistent with those of Tasić-Otašević et al.[39], who identified Candida albicans as the predominant *Candida* species in adult patients with otomycosis. The prevalence of *Candida albicans* in our region may be attributed to environmental factors or a strong tendency of this species to colonies the external auditory canal[40].

Regarding the *ERG11 gene*, it has been found to contribute to increased antifungal resistance. The ERG biosynthesis pathway is disrupted by azole compounds through inhibition of 14-alpha-sterol demethylase activity[41]. Out of one hundred and fourteen *Candida* species. (75.4%) of the isolates exhibited ergosterol resistance. *Candida albicans* showed the highest resistance rate, at (85.7%) of the isolates. Significantly high resistance was also observed in *Candida tropicalis* (78.6%) and *Candida parapsilosis* (73.7%) and moderate resistance levels were recorded in *Candida glabrata (*62.5%) and *Candida krusei* (58.3%). These findings demonstrate a significant prevalence of ergosterol resistance, as mutations in the *ERG11* gene substantially affect azole resistance by modifying the target enzyme, lanosterol 14-alpha-demethylase. The elevated resistance levels noted in *Candida albicans*, Candida tropicalis and, *Candida parapsilosis* underscore the clinical significance of assessing *ERG11*-mediated resistance to guarantee the efficacy of antifungal treatment.

CONCLUSION

Candida species represent a major cause of otomycosis infections in humans. Although azole antifungal agents remain effective in the treatment of these infections, the emergence of azole resistance has become an important clinical concern. Molecular analysis has shown that mutations in the ergosterol biosynthesis gene (*ERG11*) play a major role in the development of azole resistance among Candida species.

Ethical Approval

The study was conducted in accordance with the ethical approval granted by the Basra Health Directorate, as per protocol number EC 126, dated December 25, 2024. All study participants also gave their verbal consent.

Conflict of Interest

All authors declare that there is no conflict of interest in this article.

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