

A Comparison of Antifungal Drugs and Traditional Antiseptic Medication for Otomycosis Treatment

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ABSTRACT

Objectives: Otomycosis infections are a common problem in otolaryngology. Traditional topical and systemic antifungal treatments can be associated with resistance, mucosal irritation, and systemic toxicity. These challenges necessitate the development of new approaches and cellular targets for therapeutic intervention. **Aim:** This study investigates the prevalence of otomycosis, species distribution, predisposing factors, sex distribution and evaluates the effectiveness of non-specific antifungal agents as a safer alternative to conventional antifungal medications in treating otomycosis. **Method:** Ear swabs were collected from 114 patients with otomycosis attending the otolaryngology clinic at two main hospitals. Yeast identification was done based on the microscopical and morphological characteristics of the colony. The antifungal efficacy of the tested drugs was assessed using an agar well-diffusion assay, and molecular methods were employed to identify the yeasts. **Results:** The results showed that females were more affected (54.38%) than males (45.61%). The most prevalent species identified was *Candida albicans* (36.8%), followed by *Candida parapsilosis* (26.3%), *Candida glabrata* (14%), and *Candida tropicalis* (12.4%), while *Candida krusei* had the lowest prevalence (10.5%). with the highest prevalence recorded in the 31-40 age group (20.2%). Four nonspecific topical antifungal agents for the ear, povidone iodine (7.5%) and hydrogen peroxide (3%), showed the strongest inhibitory activity against *Candida* species isolates, while acetic acid (2%) and isopropyl alcohol (70%) demonstrated weaker effects. **Novelty:** Topical antiseptic agents are regarded as effective alternatives or adjuncts to antifungals for the treatment of ear infections, particularly otomycosis. Antiseptics serve to minimize fungal infection, restrict microbiological growth, and prevent the development of antifungal resistance.

INTRODUCTION

Otomycosis is a superficial fungal infection of the external auditory canal (EAC) that is increasingly common in otolaryngology (ENT) clinics [1,2]. It may present as an acute, subacute, or chronic illness and is a globally prevalent infection, with incidence varying across geographic regions [3-5]. The prevalence ranges from about 9% of cases of otitis externa to 30% in patients with symptoms of otitis or inflammatory ear conditions [6]. The factors contributing to the development of otomycosis are categorized into environmental and host-derived. The warm and humid climate typical of tropical and subtropical zones represents the most significant external risk factor [7,8]. Host-derived risk factors include excessive earwax production, minor infections, local trauma and physical injury to the ear canal, use of occlusive hearing aids, immunocompromised status, diabetes, self-cleaning with cotton swabs, and swimming [9,10]. Otomycosis may also develop following bacterial infections of the EAC treated with topical antibacterial agents, increased use of topical antibiotics/steroids, or

chemotherapeutic agents. Additionally, the disease may result from autoinfection of the canal in patients with untreated cutaneous otomycosis [11,12]. The main clinical manifestations in most otomycosis patients are pruritus, occasional otalgia, aural fullness, and hearing loss [13,14].

Treatment methods for ear fungus include removing the causative agent, thoroughly cleaning the ear canal, and administering antifungal medications, including specific antifungal agents (e.g., azoles) and nonspecific antifungal agents [15,16]. However, the treatment of mycotic infections is becoming increasingly challenging due to the global rise in antifungal resistance [17,18]. The primary factor driving antifungal resistance is the misuse or overuse of antifungal drugs, which promotes selective pressure and adaptation in fungal populations [19]. Additionally, the dosages and antimicrobial spectra of these agents can significantly influence the human microbiome [20]. This study aims to evaluate the antifungal properties of common antiseptic agents to identify an effective and low-cost alternative for treating ear fungal infections. Previous studies have demonstrated promising antifungal effects of antiseptics in the management of otomycosis and evaluating their applicability in our population and comparing their efficacy and safety with that of 1% clotrimazole ear drops.

RESEARCH METHOD

Clinical examinations and samples collection

The study was conducted over a 12 month, from January to December 2025. Ear swabs were obtained from patients attending the ENT Department at Al-Basrah Teaching Hospital and Al-Sadr Teaching Hospital and then transferred to the Mycology Laboratory at the College of Science, University of Basrah, Iraq. All patients presenting with symptoms of otomycosis were examined by an ear, nose, and throat (ENT) specialist. Ear swabs were collected from 114 patients aged 7 to 75 years. The isolates were categorized by patient sex. Furthermore, the isolates were categorized by the following patient age groups (in years): 7-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, and 71-80, and all symptoms, such as discharge, itching, inflammation, and hearing impairment, were recorded in a questionnaire. Isolation, identification, and characterization were performed according to morphological criteria. Samples were collected using sterile swabs and cultured on CHROMagar media and Sabouraud dextrose agar (SDA) (Himedia, India), supplemented with chloramphenicol [21,22], and incubated at 37°C for 2-4 days. Direct smears were stained using the lactophenol cotton blue and examined by microscopy [23].

The antiseptic agents

Different concentrations of non-specific ototopical antifungal agents were prepared from the original stock solution, as described by Mary et al. [24], where 3% H₂O₂ was prepared from 50% H₂O₂, 7.5% povidone iodine was prepared from 10% iodine, 70% isopropyl alcohol was prepared from 99.5% isopropyl alcohol and 2% acetic acid was prepared from 98% acetic acid. Distilled water is used as a diluent solvent to prepare different concentrations of non-specific ototopical antifungal agents.

Antifungal activity test

Agar well diffusion assay

The surfaces of Sabouraud dextrose agar were cultured with a suspension of actively growing yeast cells. Suspensions of approximately 6×10^6 yeast cells per ml in sterile normal saline, in comparison with McFarland tubes, were prepared and 1.5 ml of it was uniformly seeded on nutrient media in 12x1.2 cm glass Petri dishes, and the extra suspension was then drained and discarded correctly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. A predetermined volume of 1% clotrimazole, 3% H₂O₂, 1% povidone-iodine, 70% isopropyl alcohol, and 2% acetic acid, were loaded in the wells using sterilized dropping pipettes, and diffusion was allowed at room temperature for 2 hours. The plates were incubated aerobically at 35°C for 24-48 hours. Antifungal activities were determined by measuring the diameter of the inhibition zone (DIZ) in mm. Every experiment was repeated three times, and the average values of antifungal activity were calculated [25].

DNA extraction, amplification, and sequencing

DNA was extracted using the Presto™ Mini g DNA Extraction Yeast Kit (Geneaid, Taiwan) according to the manufacturer's protocol. The extracted genomic DNA was subjected to electrophoresis in a 2% agarose gel and then stained with ethidium bromide as described by Jordan and Dalmasso [26]. A 25 µL polymerase chain reaction (PCR) formulation was prepared using 12.5 µL of Go Taq Green Master Mix (2X), 1 µL of forward primer, 1 µL of reverse primer, 3 µL of DNA template, and 7.5 µL of nuclease-free water. The internal transcribed spacer regions (ITS1-5.8s-ITS4) of ribosomal DNA were amplified via PCR using 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'), according to Mirhendi et al. [27]. The thermal cycle conditions of PCR included a 1-minute initial denaturation at 95 °C, followed by 35 cycles of 1-minute denaturation at 94 °C, 45 seconds of annealing at 56 °C, and 1-minute extension at 72 °C, plus a final extension at 72 °C for 5 minutes. The amplicons were qualified by 2% agarose gel electrophoresis at 100 V and 70 mA for 45 minutes. Macrogen (South Korea), through its website <http://dna.macrogen.com>, sequenced the polymerase chain reaction (PCR) products. Yeast species were identified using the Basic Local Alignment Search tool (BLAST), which is used by the National Centre for Biotechnology Information (NCBI) <http://www.ncbi.nlm.nih.gov>. The nucleotide sequences were copied and pasted into the BLAST search tool after verification, and the program then identified the yeast species by comparing their sequences with those of other species [28].

Statistical analysis

Statistical analysis data were entered for analysis using Statistical Package for Social Sciences (SPSS) software version 23. Descriptive statistics were presented as frequencies, percentages, means, and standard deviations (SD). Categorical variables were analyzed using the chi-square test. A p-value less than 0.05 is considered statistically significant.

RESULTS AND DISCUSSION

Results

Ear swabs were collected from 114 patients aged 7 to 75 years. The isolates were categorized by patient sex, with 62 (54.4%) females and 52 (45.6%) males. The distribution of otomycosis isolates across age groups and sexes showed a heterogeneous pattern, with the highest prevalence recorded in the 31-40 age group (20.2%), followed by the 41-50 age group (18.4%) and the 51-60 age group (16.7%). As well, Infection was less frequent in younger age groups 7-20 years (11.4%) and elderly individuals over 70 years (7.9%), indicating that middle-aged adults represent the most at-risk group. The isolates and their numbers and percentages are shown in (Table 1). The most frequent symptom was pruritus (itching), reported in 44 cases (38.6%), otalgia (pain) was the second most common symptom, observed in 33 cases (28.9%), otorrhea (discharge) occurred in 23 patients (20.2%), ear fullness was recorded in 10 cases (8.8%), and Tinnitus was the least common symptom, present in 4 patients (3.5%), as shown in (Table 2).

Table 1. Distribution of otomycosis patients according to sex and age groups

Age groups (years)	Female		Male		Total	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
(7-10)	2	3.2	2	3.8	4	3.5
(11-20)	5	8.1	4	7.7	9	7.9
(21-30)	8	12.9	5	9.6	13	11.4
(31-40)	12	19.4	11	21.2	23	20.2
(41-50)	11	17.7	10	19.2	21	18.4
(51-60)	10	16.1	9	17.3	19	16.7
(61-70)	9	14.5	7	13.5	16	14.0
(71-80)	5	8.1	4	7.7	9	7.9
Total	62 (54.4%)	100	52 (45.6%)	100	114	100

Table 2. The main presentation of the patients studied

Symptom	No. of cases	Percentage
Pruritus (Itching)	44	38.6%
Otalgia (Pain)	33	28.9%
Otorrhea (Discharge)	23	20.2%
Ear fullness	10	8.8%
Tinnitus	4	3.5%
total	114	100%

($\chi^2 = 46.965$, $df = 4$, $p < 0.001$). (p-value) = 1.55×10^{-9}

Effect of non-specific ototopical antifungal agents against *Candida* species

Among the evaluated agents, 7.5% iodine demonstrated the highest overall effectiveness, producing inhibition zones of 10 ± 1.5 mm and affecting 87.7% of the isolates. Hydrogen peroxide (3%) also exhibited a good antifungal effect, with a mean inhibition zone of 10 ± 2.5 mm and activity against 70% of isolates. In contrast, isopropyl alcohol (70%) and acetic acid (2%) produced smaller inhibition zones (7 ± 1.5 mm) and affected only 52% and 38% of the isolates, respectively. Clotrimazole (1%), a specific azole antifungal agent, showed the largest inhibition zone (25 ± 4.0 mm). Nevertheless, only 43.8% of isolates were susceptible. (Table 3), (Fig. 1).

Table 3. The inhibition zone diameter of non-specific antifungal agents and 1% clotrimazole against *Candida* species on the SDA media

Non-specific antifungal agents	Diameter of inhibition zone (mm) (M \pm SE)	No. of inhibited isolates / Total (%)
7.5% Povidone Iodine	10 \pm 15 mm	100/114 (87.7%)
3% Hydrogen peroxide (H ₂ O ₂)	10 \pm 25 mm	80/114 (70%)
70% Isopropyl alcohol	7 \pm 15 mm	60/114 (52%)
2% Acetic acid	7 \pm 15 mm	55/114 (38%)
1% Clotrimazole	25 \pm 40 mm	50/114 (43.8%)

($\chi^2 = 55.362$, df = 4, p < 0.001)

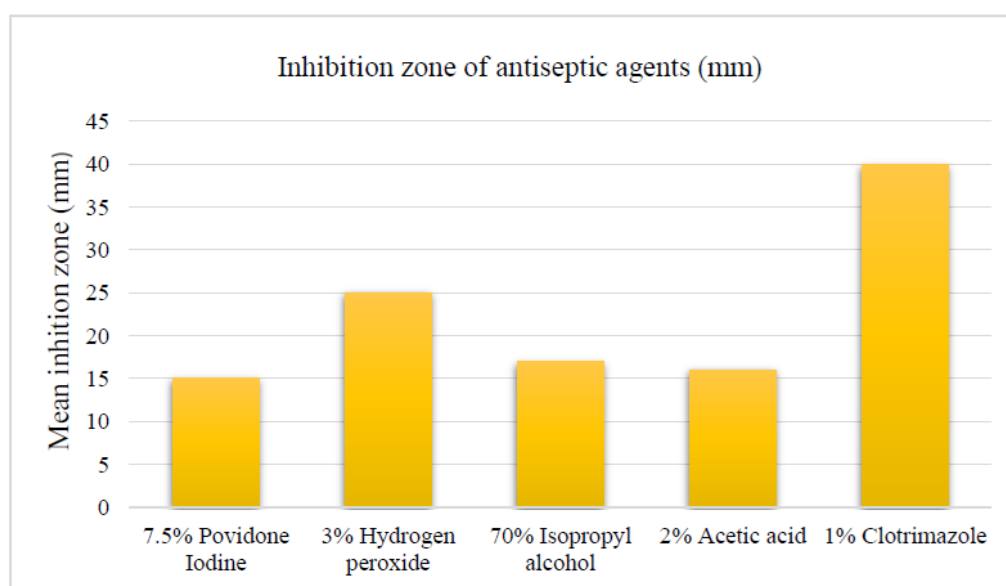


Figure 1. Inhibition zone diameter (well-diffusion method) of antiseptic agents: 2% acetic acid, 7.5% povidone iodine, 3% H₂O₂, and 70% isopropyl alcohol compared to 1% Clotrimazole.

Identification of *Candida* species on CHROMagar media

Candida species were identified using CHROMagar medium, where *Candida albicans* (36.8%), which formed characteristic light smooth green colonies, *Candida parapsilosis* (26.3%), producing white to pale pink colonies, *Candida glabrata* (14%), which

formed in white to light purple colonies, *Candida tropicalis* (12.4%), which appeared in steel-blue colonies and *Candida krusei* (10.5%), which appeared in purple-pink colonies.

Molecular identification

Molecular analysis using the internal transcribed spacer regions (ITS1-5.8s- ITS4) sequencing revealed considerable variation in the distribution of *Candida* species among clinical isolates. The most prevalent species identified was *Candida albicans* (36.8%), followed by *Candida parapsilosis* (26.3%), *Candida glabrata* (14%), and *Candida tropicalis* (12.4%), while *Candida krusei* had the lowest prevalence (10.5%), (Table 4).

Table 4. Molecular identification of *Candida* species

<i>Candida</i> species	Total No. of isolates 114	Percentage (%)
<i>Candida albicans</i>	42	36.8%
<i>Candida parapsilosis</i>	30	26.3%
<i>Candida glabrata</i>	16	14%
<i>Candida tropicalis</i>	14	12.4%
<i>Candida krusei</i>	12	10.5%

($\chi^2 = 28.98$, $df = 4$, $p < 0.001$).

Discussion

Otomycosis is a common problem in otolaryngology (ENT) practice. Treating otomycosis presents a challenge for both the patient and the ENT physician [29,30]. The present study, shows that both females (54.4%) and males (45.6%) were affected, suggesting that otic candidiasis is not strongly sex-specific. However, females showed a slightly higher prevalence in most age groups, particularly between 31 and 50 years of age, a finding consistent with several other studies [31]. This may be attributed to the traditional headscarf, which is associated with the prolonged covering of the external auditory canal. Besides, the use of a dryer in the washing and setting of hair by women also increases the humidity in the external auditory canal, and this encourages otomycosis [32,33]. The distribution of *Candida* species isolates across age groups and sexes showed a heterogeneous pattern. These results indicate that *Candida*-associated otomycosis is most common among adults in their third to sixth decades of life, possibly due to higher exposure to environmental factors, occupational conditions, or the frequent use of ear drops and self-cleaning habits that may alter the ear canal microenvironment. The epidemiology of mycotic ear infections differs among different populations and countries. Nevertheless, it is obvious that the incidence of these infestations remains higher in warm and humid areas and during the hot season [29].

The current study showed that itching was the most common symptom, occurring in approximately 38.6% of patients, suggesting that itching is the primary symptom of fungal ear infections resulting from irritation of the external auditory canal, followed by ear pain (28.9%). Moreover, ear discharge was observed in 20.2% of cases, likely due to fungal remnants or epithelial sloughing. While 8.8% of patients experienced a feeling of

fullness in the ear, possibly due to obstruction caused by masses or fungal remnants. Tinnitus was the least reported symptom, affecting only about 3.5% of patients. The low incidence of ear fullness and tinnitus may reflect that middle or inner ear infections were less common among the patients studied. Thus, it is likely that most cases originated from conditions affecting the external ear, rather than deeper auditory structures. A previous study on otomycosis by Philip *et al.*, showed that itching (76.5%) was the most common clinical manifestation, followed by ear discharge (55.9%), ear pain (52.9%), tinnitus (29.4%), and hearing loss (20.6%) [34]. Aremu *et al.*, also reported that itching (73%) was the most common clinical symptom, followed by ear pain (66.5%), ear discharge (19.6%), tinnitus (11.6%), and hearing loss (9.8%) [35]. This is similar to what was observed in our study.

In clinical practice, Clotrimazole (1%), a specific azole antifungal agent, showed the largest inhibition zone (25 ± 4.0 mm), reflecting strong antifungal activity. Nevertheless, only 43.8% of isolates were susceptible, suggesting the presence of resistant *Candida* species, particularly *Candida glabrata* and *Candida krusei*, which are known to exhibit reduced sensitivity to azole compounds. A study by Gonçalves *et al.*, found that the prevalence of azole resistance was 3.2%, which was associated with worse patient outcomes and explained that the frequent use of azole antifungals may be a significant contributing factor to the global increase in resistance [36]. Also, Van der Linden *et al.*, found statistically significant resistance to azole antifungals in otomycosis [37]. As well, a study by Kiakojuri *et al.*, indicated a decrease in relapse rates with a prolonged course of clotrimazole ear drops [38].

Antiseptic agents are frequently used for topical treatment and have a low potential for side effects. Ear sterilization is an essential first step in the treatment of ear fungus. The tested non-specific antifungal agents showed variable inhibitory effects against *Candida* isolates, as reflected by the differences in inhibition zone diameters and the percentage of affected isolates. Among the evaluated antiseptic agents, 7.5% povidone iodine demonstrated the highest overall effectiveness, producing inhibition zones of 10 ± 1.5 mm and affecting 87.7% of the isolates. This strong activity can be attributed to iodine's ability to denature proteins and disrupt cellular membranes and interfere with the enzymatic systems of microorganisms, leading to cell death [39]. 70% Isopropyl alcohol and 2% acetic acid produced smaller inhibition zones (7 ± 1.5 mm) and affected only 52% and 38% of the isolates, respectively, indicating limited antifungal potency. Acetic acid is used to treat superficial infections of the external auditory canal caused by organisms susceptible to antibiotics, and no toxic effects have been reported with an overdose of 2% acetic acid in ear solution [11,40]. The latest guidance from the National Institute for Health and Care Excellence (NICE) recommends the promotion of self-care with over-the-counter acetic acid ear drops or spray [41]. While the results of 3% hydrogen peroxide exhibited a good antiseptic effect, with a mean inhibition zone of 10 ± 2.5 mm and activity against 70% of the isolates. Hydrogen peroxide acts as an oxidizing agent by producing free radicals that react with lipids, proteins, and nucleic acids, affecting cellular components in a

nonspecific manner [42]. In this practice, the antiseptic effect of hydrogen peroxide on SDA media was lower than that of povidone iodine. This may have resulted from the low hydrogen peroxide concentration used, or this fungus may require a hydrogen peroxide concentration higher than 3% to prevent its growth. As well, the antifungal activity of 3% hydrogen peroxide against *Candida species* was stronger than that of 2% acetic acid. This may be due to *Candida's* inability to resist hydrogen peroxide because it cannot survive in the presence of oxygen [43].

Several methods are used to identify *Candida* species in clinical laboratories. Traditional morphological and biochemical methods are gradually being replaced by newer molecular approaches, allowing for faster and more accurate identification [44,45]. Our results showed that Chromoagar medium performed at an average level in identifying *Candida* species in clinical laboratories. In fact, the sensitivity and specificity of Chromoagar medium in detecting both *Candida albicans* and *non-Candida* species were acceptable. The species identification was confirmed by the molecular detection through the internal transcribed spacer regions (ITS1-5.8s-ITS4) by PCR, which proved to be a valid and sensitive technique [46].

CONCLUSION

Fundamental Finding : Topical antiseptic agents are regarded as effective alternatives or adjuncts to antifungals for the treatment of ear infections, particularly otomycosis. **Implication** : Antiseptics serve to minimize fungal infection and restrict microbiological growth. **Limitation** : The statement does not specify clinical conditions, dosage, or comparative effectiveness across different antiseptic agents. **Future Research** : Further investigation is needed to evaluate their role in preventing the development of antifungal resistance.

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