

A search for new otomycotic species and their sensitivity to different antifungals

MOHAMMED RIFAAT AHMED^{1,*}, ASHRAF SAAD ABOU-HALAWA¹,
WAHEED F. HESSAM², DIAA' SALAHELDIN ALY ABDELKADER³

¹Faculty of Medicine, Department of Otolaryngology, Suez Canal University, Ismailia, Egypt

²Faculty of Medicine, Department of Microbiology and Immunology, Suez Canal University, Ismailia, Egypt

³Resident of Otolaryngology, Al-agouza Police Hospital, Al-agouza, Egypt

*Corresponding author: Mohammed Rifaat Ahmed; Faculty of Medicine, Department of Otolaryngology, Suez Canal University, Ismailia 41511, Egypt; Phone: +20 12 85043825; Fax: +20 66 3415603; E-mail: m_rifaat@hotmail.com

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Abstract: *Background:* Otomycosis is a common ear problem in countries with hot and humid climate. Emergence of new fungal species is a possibility particularly in patients with chronic illness or who receives antibiotics either systemically or topically. *Aim:* To identify the otomycotic species, which are responsible for developing the otomycosis. *Methods:* A descriptive study was carried out in 63 patients who were clinically diagnosed with otomycosis. Swabs were taken from the fungal debris to identify the causative agent and to determine the effective antifungals against it. *Results:* *Aspergillus* species were the most common agents and were found in 47 patients (74.6%), represented by *A. fumigatus* (36.5%), *A. niger* (27%), *A. flavus* (6.5%), and *A. terreus* (4.8%). Ketoconazole (90%) and miconazole (76%) were the most effective antifungals against the species obtained from cultures, whereas fluconazole was the least effective. *Conclusions:* Although there is no change in the mycology of otomycosis, there is still a significant variability in the isolated species and in their sensitivity to antifungal drugs. Ketoconazole exhibited broad spectrum effect against fungal isolates in this study, whereas fluconazole was the least efficacious.

Keywords: otomycosis, culture, antifungals, treatment, etiology

Introduction

Otomycosis, which is a fungal infection that affects the external ear canal, may involve the middle ear in case of a perforated tympanic membrane [1]. Itching is the most prevalent symptom present in more than 90% of the patients. Some patients may have superimposed bacterial infection with pain, hearing loss, and tinnitus [2].

Otomycosis is particularly frequent in hot and humid regions (as high as 54%), but the prevalence decreases to 9% in temperate climate. Swimming was reported as a risk factor for otomycosis. Topical antibiotics in the ear canal have also been reported as a predisposing factor for otomycosis [3, 4].

The prevalence of dermatomycoses in patients with otomycosis ranged from 36.5% to 51% and the same pathogenic fungi were isolated from dermatomycoses

and otomycosis in nearly half of these patients. However, the knowledge on otomycosis is still limited [5, 6].

Identification of correct pathogenic organism and predisposing factors makes it easier for the clinician to find suitable treatment and to prevent recurrence [7].

This study aimed to identify the otomycotic species and the antifungals to which they are sensitive, among patients who were clinically diagnosed with otomycosis and to look for new otomycotic species.

Patients and Methods

This study was performed in Otorhinolaryngology and Microbiology departments in Suez Canal University Hospital, in the period from June 2016 to September 2016 to assess the spectrum of the otomycotic species and their sensitivity to different antifungals.

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A total of 63 consecutive patients were included in the study based on clinical diagnosis of otomycosis through typical symptoms, mainly itching sensation, otalgia, and ear discharge, and otoscopic findings of fungal mass or debris.

We excluded the patients who had other ear problems, for example, those with perforated tympanic membrane and chronic otitis media and the patients who were treated for otomycosis recently.

All patients were subjected to brief history, including name, age, gender, occupation, presence of risk factors, otoscopic examination (to confirm the diagnosis), and sample collection (using sterile cotton swabs) from the external ear canal.

Mycological Analysis

Direct microscopic examination

Direct smears of the fungal debris were prepared and examined using lactophenol cotton blue stain (LPCB) [8].

Mycological cultures

Swabs were streaked on Sabouraud's dextrose agar (SDA) medium with the composition of (g/l): peptone 15, dextrose 40, and agar 20 (146). Cultures were incubated at 28 °C until fungal colonies appear. The culture plates were examined daily for the presence of growth from days 3–4 [8].

Phenotypic identification

Fungi were identified on the basis of macro- and microscopic features, i.e., morphology of the colonies and microscopic examination of fungal smear stained with LPCB.

In vitro sensitivity to antifungal agents

Disc diffusion method was employed, but SDA medium was used instead of Mueller-Hinton II agar [8]. Inhibition zone around each disc was measured in millimeters and the fungal isolates were classified as sensitive, intermediate, or resistant. The used antifungals were fluconazole, ketoconazole, itraconazole, miconazole, and nystatin.

Patients were treated with the most effective antifungal identified through the culture and the sensitivity analysis. After debridement of the external auditory canal, a gauze wick impregnated with the selected antifungal was

inserted into the external auditory canal. The patient was asked to come back after 3 days and a new wick was inserted.

Statistical analysis

The statistical analysis of this study was carried out using Statistical Program for Social Science (SPSS, version 15, Chicago, IL). χ^2 test was used for statistical interpretation. The *p* values below 0.05 were considered significant.

Ethics

Written consent was obtained from each patient before including him in the study. Local ethics committee has approved the study.

Results

A total of 63 patients diagnosed with otomycosis (by history and otoscopic examination) were included in the study. Swabs were taken from the fungal debris and sent for microbiological analysis to identify the causative organism and its sensitivity toward different antifungals. About 34 cases (54%) were males and 29 cases were females (46%) with mean age of 41.3 ± 7.4 years. Majority of patients (90.48%) had unilateral otomycosis.

The most common predisposing factors for otomycosis are summarized in *Table I*. Self-inflicted trauma by either matchstick or cotton bud to relieve the itch and ear wash were found in 23 patients (36%). Swimming in potentially contaminated water was reported by 17 cases (27%). Chronic diseases impairing the immunity like diabetes mellitus and prolonged use of antibiotics were found in few cases.

Fungal identification through mycological culture and fungal smear was performed. The most common fungal isolates were *A. fumigatus* in 23 cases (37%), *A. niger* in 17 cases (27%), and *Candida albicans* in 13 cases (21%). *A. flavus* was isolated in four cases (6%), *Aspergillus*

Table I Most common predisposing factors to otomycosis among study group

Predisposing factor	No. of patients	Percentage (%)
Swimming	17	27
Associated chronic diseases	13	21
Self-inflicted trauma to auditory canal	23	36
Prolonged use of antibiotics/ immunosuppressives	10	16

Table II | Causative agent to otomycosis in the study population

Causative agent	No. of patients	Percentage (%)
<i>Aspergillus niger</i>	17	27
<i>Aspergillus fumigatus</i>	23	37
<i>Aspergillus flavus</i>	4	6
<i>Aspergillus terreus</i>	3	5
<i>Candida albicans</i>	13	21
<i>Candida tropicalis</i>	3	5

terreus in three cases (5%), and *Candida tropicalis* in three cases (5%) (Table II). There is no significant difference between males and females according to the identified fungal species (Table III).

Efficacy of a variety of antifungals against species obtained from cultures was tested. The used antifungals

were fluconazole, ketoconazole, itraconazole, miconazole, and nystatin (Table IV). Ketoconazole had the highest efficacy profile against the fungal species obtained. It was efficacious in 57 cases (90%), but resisted in only two cases (2%). Miconazole came next in the efficacy profile, where it was sensitive to 76% of the tested isolates. Overall, ketoconazole and miconazole were the most efficacious antifungal against *Aspergillus* species. Fungal isolates showed resistance to fluconazole in 23 cases (nearly 37%).

The other antifungals showed variable degrees of efficacy, that is, Nystatin had a relatively good effect against *Candida* species, as it was sensitive against 12 of 16 cases caused by this species.

Duration of treatment was 1 week. The proportion of antifungals used were as follows: ketoconazole 2% cream in 23 cases, miconazole 2% cream in 20 cases, and nystatin 100,000 IU/gm cream in 20 cases. After 2 weeks of treatment, all patients had relief of symptoms

Table III | Causative agents of otomycosis in relation to gender

Causative organism	Male		Female		χ^2	p value
<i>Aspergillus niger</i>	9	14%	8	12.7%	0.13	0.18 NS
<i>Aspergillus fumigatus</i>	11	18%	12	19%	0.24	0.05 NS
<i>Aspergillus flavus</i>	3	5%	1	1.5%	0.34	0.21 NS
<i>Aspergillus terreus</i>	2	3%	1	1.5%	0.12	0.07 NS
<i>Candida albicans</i>	7	11%	6	9.5%	0.29	0.08 NS
<i>Candida tropicalis</i>	2	3%	1	1.5%	0.31	0.09 NS

There was no statistically significant difference ($p > 0.05$). NS: not significant

Table IV | Sensitivity of otomycotic fungal isolates to different antifungal agents

Fungus isolate	DS	Fluconazole	Ketoconazole	Itraconazole	Miconazole	Nystatin
<i>Aspergillus niger</i> (17 cases)	S	5(29%)	15(90%)	6(35%)	15(90%)	4(24%)
	I	4(24%)	1(5%)	4(24%)	1(5%)	3(18%)
	R	8(47%)	1(5%)	7(41%)	1(5%)	10(58%)
<i>Aspergillus fumigates</i> (23 cases)	S	5(22%)	21(92%)	18(39%)	12(52%)	12(52%)
	I	7(30%)	1(4%)	3(43%)	10(44%)	6(26%)
	R	11(43%)	1(4%)	2(18%)	1(4%)	5(22%)
<i>Aspergillus flavus</i> (4 cases)	S	2(50%)	4(100%)	3(75%)	4(100%)	3(75%)
	I	1(25%)	0(0%)	1(25%)	0(0%)	1(25%)
	R	1(25%)	0(0%)	0(0%)	0(0%)	0(0%)
<i>Aspergillus terreus</i> (3 cases)	S	1(33%)	2(66%)	1(33%)	2(66%)	0(0%)
	I	1(33%)	1(33%)	1(33%)	1(33%)	1(33%)
	R	1(33%)	0(0%)	1(33%)	0(0%)	2(66%)
<i>Candida albicans</i> (13 cases)	S	10(77%)	12(92%)	11(85%)	12(92%)	10(54%)
	I	2(15%)	1(8%)	1(7.5%)	1(8%)	2(23%)
	R	1(8%)	0(0%)	1(7.5%)	0(0%)	1(23%)
<i>Candidatropicalis</i> (3 cases)	S	1(33%)	3(100%)	1(33%)	3(0%)	2(66%)
	I	1(33%)	0(0%)	1(33%)	0(0%)	1(33%)
	R	1(33%)	0(0%)	1(33%)	0(0%)	0(0%)
Total isolates (63 cases)	S	24(38%)	57(90%)	40(64%)	48(76%)	31(50%)
	I	16(25%)	4(6%)	11(17%)	13(21%)	13(20%)
	R	23(37%)	2(4%)	12(19%)	2(3%)	19(30%)

DS: degree of sensitivity; S: sensitive; I: intermediate; R: resistant

and otoscopic examination showed normal skin and no visible fungal debris.

Discussion

In this study, males are slightly more affected with otomycosis than females, i.e., 34 cases (54%) out of 63 patients. Moharram et al. [9] and Abdelazeem et al. [10] also had more males in their studies who were affected with otomycosis (60.9% and 63.6%, respectively). This male preponderance may be explained by the fact that males in our community have more outdoor activities than females. Therefore, it increases the exposure to humidity and dust that carry fungal spores. In addition, more males go for swimming than females.

Traumatic injury to the auditory canal comes first in the predisposing factors (36%), followed by swimming in the potentially contaminated water (27%). These findings are similar to the studies of Abdelazeem et al. [10] and Jia [11]. In both the studies, external ear injury was the main predisposing factor in 70% and 72% of their patients, respectively [10, 11]. Violent manipulation to the ears by the patient using either his finger tips or match sticks to clean the ear or to relieve the sensation of itching may lead to infiltration of the skin with fungal debris and infection. Rubbing the ears with feathers, matchstick, and contaminated finger tips are known to encourage the inoculation and growth of the spores of fungus on the moist external auditory canal especially in patient with poor personal hygiene [12].

In a study from Indian subcontinent, Prasad et al. had demonstrated that the most common predisposing factor was instillation of coconut oil in the ears (42%), as many people think that it is beneficial to the ears. Paradoxically, its sporostatic effect may help to maintain the viability of fungal conidia [13].

In this study, out of 63 patients, *Aspergillus* species have the upper hand in the disease pathogenesis in 47 cases (75%), followed by *Candida* species in 16 cases (25%). From Iran, Saki et al. [14] reported their fungal isolates percentages as follows: *A. niger* (67%), *A. flavus* (13%), *C. albicans* (12%), *A. fumigatus* (6%), and *Penicillium* species (2%). Of all the 100 patients with clinically diagnosed otomycosis, Prasad et al. found that *Aspergillus* were isolated in 80% cases, in which *A. niger* complex was the most common fungal isolate (38%), followed by *A. fumigatus* complex (27%) and *A. flavus* complex (15%). *Penicillium* species (8%), *Candida albicans* (4%), *Rhizopus* spp. (1%), and *Chrysosporium* spp. (1%) were the other fungi isolated. Single fungus was isolated from 94% of cases [13].

In this study, we tested the sensitivity of fungal isolates to different antifungals: fluconazole, ketoconazole, itraconazole, miconazole, and nystatin. Ketoconazole had

the highest efficacy profile against the fungal species obtained. It was efficacious in 57 cases (90%) and was the least resistible antifungal, but it was resisted in only two cases (2%). Miconazole came next in the efficacy profile. It was efficacious against 76% of the tested isolates. Both are azoles that reduce the concentration of ergosterol, an essential sterol used by fungi to synthesize the cytoplasmic membrane [15]. However, it seems that not all the azole group members have the same efficacy against otomycosis fungal species. In this study, fungal isolates showed high resistance to fluconazole (in 37% of cases).

The other antifungals showed variable degrees of resistance. Nystatin had a relatively good effect against *Candida* species in 12 out of 16 cases. It is a polyene macrolide antibiotic that inhibits sterol synthesis in cytoplasmic membrane. Many molds and yeasts are sensitive to nystatin including *Candida* species. A major advantage of nystatin is that it is not absorbed from intact skin. Nystatin is not available as an otic solution, but it can be prescribed as cream, ointment, or powder with efficacy rates up to 50%–80% [15]. In a study by Moharram et al. [9], terbinafin and clotrimazole were the most effective agents, whereas fluconazole and itraconazole were the least effective. *Aspergillus* species showed high resistance to fluconazole. Resistance to antifungal drugs may be more in the presence of risk factors, as recurrent external auditory canal injury during self-cleaning with sharp objects used to relieve the itching sensation. Bad control of diabetes mellitus and repeated and prolonged use of topical antibiotic and steroid drops may also lead to resistant strains development.

In this study, management included removing all visible fungal debris and selecting the most sensitive antifungal for each case according to the sensitivity test, with the priority given to topical forms. Ketoconazole 2%, miconazole 2%, and nystatin 100,000 IU creams were used and all cases showed a marked improvement 3 days and 2 weeks after treatment. As stated by Anwar et al. [16], topical antifungals should be started immediately after thorough removal of fungal debris.

Possibility of the emergence of new strains or development of pathogens' resistance to standard antimycotics on one hand and development of new techniques for cultures, development of new generations of antifungals, and changing the concept toward systemic antimycotics versus topical treatment stress the need to reevaluate our treatment plan from time-to-time.

Conclusions

Although there is no change in the mycology of otomycosis, there is still a significant variability in the isolated species and in their sensitivity to antifungal drugs. Ketoconazole exhibited broad spectrum effect against fungal

isolates in this study, whereas fluconazole was the least efficacious.

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