

Identification of Fungal Pathogens in Otomycosis and Their Drug Sensitivity: Our Experience

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Int Arch Otorhinolaryngol 2018;22:400–403.

Abstract

Introduction Otomycosis is a common problem in otolaryngology practice. However, we usually encounter some difficulties in its treatment because many patients show resistance to antifungal agents, and present high recurrence rate.

Objectives To determine the fungal pathogens that cause otomycosis as well as their susceptibility to the commonly used antifungal agents. Additionally, to discover the main reasons for antifungal resistance.

Methods We conducted an experimental descriptive study on 122 patients clinically diagnosed with otomycosis from April 2016 to April 2017. Aural discharge specimens were collected for direct microscopic examination and fungal culture. In vitro antifungal susceptibility testing was performed against the commonly used antifungal drugs. We tested the isolated fungi for their enzymatic activity.

Results Positive fungal infection was found in 102 samples. The most common fungal pathogens were *Aspergillus* and *Candida* species, with *Aspergillus niger* being the predominant isolate (51%). The antifungal susceptibility testing showed that mold isolates had the highest sensitivity to voriconazole (93.48%), while the highest resistance was to fluconazole (100%). For yeast, the highest sensitivity was to nystatin (88.24%), followed by amphotericin B (82.35%), and the highest resistance was to terbinafine (100%), followed by Itraconazole (94.12%). Filamentous fungi expressed a high enzymatic ability, making them more virulent.

Conclusion The *Aspergillus* and *Candida* species are the most common fungal isolates in otomycosis. Voriconazole and Nystatin are the medications of choice for the treatment of otomycosis in our community. The high virulence of fungal pathogens is owed to their high enzymatic activity. Empirical use of antifungals should be discouraged.

Keywords

- ▶ otomycosis
- ▶ yeast
- ▶ aspergillus
- ▶ antifungal agents
- ▶ candida
- ▶ dermatophytes

Introduction

Otomycosis is a common condition, frequently encountered in otolaryngology outpatient clinics. It is defined as a fungal infection affecting the external ear canal in the majority of cases. Less commonly, it can involve the middle ear, if the drum is perforated, and the mastoid cavity following an open mas-

toideotomy. Common associated symptoms include itching, pain, aural fullness, aural discharge, hearing impairment and tinnitus.^{1–3}

Various types of fungal species were encountered in otomycosis. However, the genus *Aspergillus* seemed to be the most common causative agent, particularly *Aspergillus niger*. Other fungal agents include species from the genera

received
October 17, 2017
accepted
December 17, 2017
published online
April 12, 2018

DOI <https://doi.org/10.1055/s-0038-1626702>.
ISSN 1809-9777.

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Penicillium, *Fusarium*, *Mucoraceae*, *Scopulariopsis*, *Alternaria*, *Malassezia*, and *Candida*, as well as various dermatophytes.⁴⁻⁶ Common risk factors for otomycosis are poor hygienic conditions; minor trauma; inflammation or physical injury; use of swimming pools; being exposed to hot humid atmospheres, as in tropical and subtropical areas; prolonged use of antibiotics, and use of steroid ear drops in those with impaired immune system.^{4,7-9}

Many antifungal agents were prescribed for otomycosis eradication, including azole group antifungals, amphotericin B, boric acid, mercurochrome (1%–2% solution) and phenylmercuric acetate (0.02%) in sterile water, urea-acetic acid solution, or aluminum acetate solution (5%).¹⁰⁻¹³ In the present study, we focused on the causative fungi for otomycosis in our region, as well as on their susceptibility to the commonly used antifungal agents. In addition, we tried to clarify the reason for newly emerged antifungal resistance.

Methods

This study fulfills the requirements of the regional and institutional ethical guidelines on studies involving human participants as well as those of the Helsinki declaration. An informed consent was obtained from all patients enrolled in this work.

Patients' selection: an experimental descriptive study was performed on patients clinically diagnosed with otomycosis who attended our otolaryngology outpatient clinic for 1 year (April 2016 to April 2017). Those patients presented with different complaints, including aural pain, itching, otorrhea, with or without hearing loss, and their examination revealed erythema, fungal debris and creamy or blackish aural discharge. Those who had recent history of antifungal topical medication were excluded from our study.

Specimen preparation & processing: After the clinical diagnosis was established, specimens from the external ear canal were collected from all patients by means of sterile cotton swabs under aseptic conditions. Each sample was divided into two parts for fungal analysis. One part was clarified with a 10% potassium hydroxide solution on a glass slide for direct microscopic examination. The second part of the specimen was mounted on the surface of two Sabouraud's dextrose agar (SDA) plates supplemented with 0.05 mg/mL of chloramphenicol (AppliChem GmbH, Darmstadt, Germany); one was incubated at 37°C and the other was incubated at 27°C. Incubation of both plates continued for at least 4 weeks with daily examination for the first week, then twice weekly for the next 3 weeks until the colonies appeared or revealed no growth.

The growing fungi were kept for further mycological testing in SDA slants and sterile Eppendorf tubes containing sterile glycerol in distilled water with a concentration of 20% for yeasts, and a concentration of 10% for filamentous fungi. In addition, Vitek-2 (bioMérieux, Marcy-L'Etoile, France) was used for complete identification of *Candida* species.¹⁴

Antifungal susceptibility testing: the antifungal susceptibility testing was performed using the disc diffusion method.¹⁵ The antifungal agents tested were: polyenes;

(amphotericin B 100 units and nystatin 100 units), azoles; (fluconazole 25 µg, ketoconazole 10 µg, clotrimazole 10 µg, voriconazole 1 µg and itraconazole 10 µg) and terbinafine 25 µg. All the antifungal discs were obtained from HiMedia, India, except for terbinafine (25 µg) and itraconazole (10 µg), which were locally prepared. The isolated yeast and filamentous fungi were tested for extracellular enzyme (urease, lipase and protease) production, according to previously described methods.^{16,17}

Statistical analysis; Data were analyzed using the STATA intercooled version 12.1 (Stata Corp. LP, College Station, TX). Quantitative data were expressed as mean, standard deviation, median and range. Qualitative data were presented as numbers and percentages.

Results

Our study included 112 patients clinically diagnosed with otomycosis. Of these patients, 102 revealed fungal pathogens. Fifty-nine of the patients were male (57.84%). Their ages ranged between 9 months and 71 years, with the highest proportion among the 21–30 years age group, median 25.7 years (27%).

The most common presenting symptom was pruritis, in 94 cases (92.16%), followed by otalgia, in 52 cases (50.98%), otorrhea, in 21 cases (20.59%), hearing loss, in 22 cases (23.53%) and tinnitus, in 10 cases (9.8%).

The causative agents for otomycosis in our study were described in ► **Table 1**. *Aspergillus niger* was the most common fungus, having been found in 49 cases (48.04%).

Antifungal Susceptibility Testing (AST) of Isolated Filamentous Fungi

Antifungal susceptibility testing of 92 mold isolates was performed using the disc diffusion method (► **Table 2**). The highest percentage of sensitivity among mold isolates was to voriconazole (93.48%), followed by terbinafine (75%). Surprisingly, all isolates were resistant to fluconazole.

Antifungal Susceptibility Testing of Isolated Yeasts

Antifungal susceptibility testing of 17 yeast isolates was performed using the disc diffusion method. For the used antifungal agents, the highest percentage of sensitivity among yeast isolates was to nystatin (88%), followed by amphotericin B (82%). All yeast isolates were resistant to terbinafine (100%) and resistance to itraconazole was observed in 94.12%.

Discussion

Otomycosis is a common clinical entity. Although not life threatening, it can be a frustrating condition for both patient and physician due to the need for a long-term therapy, regular follow-up and tendency for recurrence.¹⁸

In this study, the most common symptoms of otomycosis were pruritis (92.16%) and otalgia (50.98%). Other common complaints were hearing loss (23.53%) and ear discharge (20.59%). The mentioned complaints and their incidence were in accordance with the findings of other studies.^{19,20} However, some reports stated that otalgia was the most

Table 1 Distribution of patients with otomycosis according to isolated fungal pathogens

Microorganisms	Number (%)
<i>Aspergillus niger</i>	49 (48.04%)
<i>Aspergillus flavus</i>	31 (30.39%)
<i>Candida famata</i>	3 (2.94%)
<i>Aspergillus terreus</i>	2 (1.96%)
<i>Candida parapsilosis</i>	2 (1.96%)
<i>Candida utilis</i>	2 (1.96%)
<i>Rhizopus stolonifer</i>	2 (1.96%)
<i>Candida guilliermondii</i>	1 (0.98%)
<i>Candida krusei</i>	1 (0.98%)
<i>Cryptococcus laurentii</i>	1 (0.98%)
<i>Penicillium duclauxi</i>	1 (0.98%)
<i>A. flavus</i> + <i>C. utilis</i>	2 (1.96%)
<i>A. flavus</i> + <i>C. famata</i>	1 (0.98%)
<i>A. niger</i> + <i>C. guilliermondii</i>	1 (0.98%)
<i>A. niger</i> + <i>C. krusei</i>	1 (0.98%)
<i>A. niger</i> + <i>C. utilis</i>	1 (0.98%)
<i>A. terreus</i> + <i>Cryptococcus laurentii</i>	1 (0.98%)
Total	102 (100%)

frequent symptom.²¹ Accordingly, absence of pruritis does not exclude the possibility of otomycosis.

Previous reports have concluded that the diagnostic yield of direct microscopic examination (DME) may range from 10 to more than 90%, depending on the fungal species.²² In this study, only 10 samples were positive for fungal infection on DME (8.2%), while on SDA culture, the percentage of samples positive for fungal infection was 83.61% (102 of total collected samples). Therefore, culture is the main diagnostic tool.²²

In our study, *A. niger* (50.98%) was the most common isolated organism, followed by *Aspergillus flavus* (33.33%), and then by *Candida* species (14.7%). This is in accordance with other studies.^{23,24} However, the predominant fungal pathogens in otomycosis were different in various literature reports, including *A. flavus*^{18,25} *Aspergillus fumigates*²⁶ and *Candida*.^{5,27} We owed this difference to the variability in geographic distribution and environmental factors.

This study revealed that voriconazole and nystatin should have the upper hand in treatment of otomycosis. Moreover, it was informative enough to explain one of the main reasons for lack of response to commonly prescribed antifungals and high recurrence rate, which was recently observed in our practice with prescription of fluconazole as a first-line treatment for our cases.

Another reason is the urease activity, which was proven to play a crucial role for fungal pathogens, particularly those that initiate infection via the lungs.²⁸ Stehr et al confirmed that

Table 2 Antifungal susceptibility pattern of mold isolates against various antifungal agents

Name	No	Drug sensitivity	AP	NS	FU	IT	VOR	KT	CLO	TF
			N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
<i>A. niger</i>	52	S	48 (92.3)	29 (55.77)	0	29 (55.77)	51 (98.08)	0	0	37 (71.15)
		I	2 (3.85)	22 (42.31)	0	12 (23.08)	0	23 (44.23)	36 (69.23)	11 (21.15)
		R	2 (3.85)	1 (1.92)	52 (100)	11 (21.15)	1 (1.92)	29 (55.77)	16 (30.77)	4 (7.69)
<i>A. flavus</i>	34	S	1 (2.94)	18 (52.94)	0	17 (50)	32 (94.12)	13 (38.24)	5 (14.71)	30 (88.24)
		I	2 (5.88)	13 (38.24)	0	7 (20.59)	2 (5.88)	21 (61.76)	29 (85.29)	1 (2.94)
		R	31 (91.18)	3 (8.82)	34 (100)	10 (29.41)	0	0	0	3 (8.82)
<i>A. terreus</i>	3	S	0	0	0	0	3 (100)	0	2 (66.67)	1 (33.33)
		I	0	3 (100)	0	2 (66.67)	0	3 (100)	1 (33.33)	2 (66.67)
		R	3 (100)	0	3 (100)	1 (33.33)	0	0	0	0
<i>Rhizopus stolonifer</i>	2	S	0	0	0	0	0	0	0	0
		I	1 (50)	2 (100)	0	0	0	0	0	0
		R	1 (50)	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
<i>Penicillium duclauxi</i>	1	S	0	1 (100)	0	0	0	0	0	1 (100)
		I	0	0	0	0	0	0	0	0
		R	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
Total	92	S	49 (53.26)	48 (52.17)	0	46 (50)	86 (93.48)	13 (14.13)	7 (7.61)	69 (75.00)
		I	5 (5.43)	40 (43.48)	0	21 (22.83)	2 (2.17)	47 (51.09)	66 (71.74)	14 (15.22)
		R	38 (41.3)	4 (4.35)	92 (100)	25 (27.17)	4 (4.35)	32 (34.78)	19 (20.65)	9 (9.78)

Abbreviations: AP, amphotericin; CLO, clotrimazole; FU, fluconazole; I, intermediate; IT, itraconazole; KT, ketoconazole; NS, nystatin; R, resistant; S, sensitive; TF, terbinafine; VOR, voriconazole.

Candida lack the urease enzyme, and urea is instead metabolized by a urea amidolyase.²⁹ In our study, 93.39% of isolated filamentous fungi and *Cryptococcus* were urease-positive. All *Candida* isolates were urease-negative. In addition, Yike mentioned the fact that the external digestion of protein substrates by secreted proteases is an essential requirement for survival and growth of both saprophytic and pathogenic fungal species.³⁰ In our study, 31.52% of mold isolates were high enzyme producers, 20.65% were intermediate enzyme producers, 46.74% were low enzyme producers and only 1.09% were non-enzyme producers. For isolated yeasts, 64.71% of isolates were low enzyme producers, 17.65% of isolates were intermediate enzyme producers and 17.65% of isolates were non-enzyme producers. These results might explain the high virulence and resistance of mold isolates to the commonly prescribed antifungals as well as the high rate of recurrence. Moreover, our results clarify how important the in vitro antifungal susceptibility testing is. Filamentous fungi possess a high enzymatic ability, which makes them more virulent, and the infections caused by these fungi are more aggressive. Our recommendation is to avoid the empirical prescription of antifungal drugs. If in vitro antifungal susceptibility testing cannot be routinely done, fluconazole should be discarded for treatment of otomycosis for these types of patients.

Conclusion

This study revealed that *Aspergillus* and *Candida* species were the most common fungal pathogens causing otomycosis. *Aspergillus niger* is the predominant fungal isolate.

Mold isolates showed highest sensitivity to Voriconazole, while the highest resistance was to Fluconazole. For yeast isolates, the highest sensitivity was to Nystatin, and the highest resistance was to terbinafine. Filamentous fungi possess a high enzymatic ability, which makes them more virulent, aggressive and resistant to treatment. Empirical use of antifungals in otomycosis should not be a routine practice.

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