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Evaluating the Etiological Agents Causing Otomycosis and Their Susceptibility to Various Anti-Fungal Agents

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ABSTRACT

Introduction: Otomycosis, a subacute or acute fungal ear infection, is a frequently encountered disease in otolaryngology clinics in India. It initiates with intense itching and then usually progresses to pain, hearing loss, and possibly perforations of tympanic membrane. A wide range of fungal agents are responsible for this condition.

Aim: To observe the changing trends in the etiological agents causing otomycosis and to detect their responsiveness to various commonly employed anti-fungal medications.

Methods: A single center, prospective study is conducted for two years on 956 patients presenting with itching ear along with other symptoms of otomycosis who were found to be culture positive upon examination. Antifungal susceptibility testings were performed using Disk diffusion test and Candifast kit.

Results: A. niger was found to be the predominant culprit with 36% of cases infected with it. Sensitivity test revealed that A. niger, A. fumigatus and A. terreus were all highly susceptible to Amphotericin B while A. flavus exhibited a 6.3% resistanc.. C.albicans and C.glabarta demonstrated 18.8% and 47.3% resistance against fluconazole but were highly responsive to all the other antifungal agents in the kit.

Conclusion: The most prominent pathological agent of Otomycosis is Aspergillus Niger. Over the last few decades, A. flavus has become a more common pathogen than A.fumigatus in the otomycosis. There exists high sensitivity to Amphoterecin B by A.niger, A.fumigatus, A.terreus but a 6.9% resistance by A.flavus. The predominant candida species (c. albicans) exhibits susceptibility to all the drugs except for fluconazole.

Keywords: oral pregabalin, oral clonidine, direct laryngoscopy, intubation, pressor response.

INTRODUCTION

Otomycosis, also referred to as Fungal Otitis Externa, has generally been defined as fungal infection of the outer ear canal which rarely manifests into middle ear complications. One of the most commonly diagnosed entity in ENT clinics, otomycosis is a challenging and frustrating condition, though rarely life threatening, since it usually requires long-term medical treatment along with frequent follow-ups and recurrences. It is global in distribution; however, geographical locations experiencing high temperature, humidity and dust exhibit greater prevalence. [1,2,3,4]. The prevalence of the disease

has changed from 9 percent [5]among patients presenting with signs and symptoms of otitis externa in 1985 to 94% in 2017. [6]. Otomycosis has been found to be a common medical condition in India. [8]. While some studies reveal higher prevalence in males[9,4], others have found a greater ratio of females presenting it with most research indicating that the maximum number of patients fall into the age group of 18-39 years.[8]Approximately 60 species of fungi are involved in otitis externa, however, Aspergillus Niger, Candida albicans, Actinomyces, Tryptophyton, Aspergillus Fumigatus and Candida tropicali are the most commonly isolated causative agents of otomycosis. [8]. In this article, we study the change in the trends of the etiological agents causing otomycosis along with their sensitivity towards a variety of anti-fungal treatments.

MATERIALS AND METHODS:

A Study group comprising of 956 participants, presenting with complaints of itchy earache were selected from patients attending the ENT outpatient department during a course of two years starting January 2019 to December 2020. Relevant data including age, sex, occupation, social class, underlying diseases, duration of the complaints, nature of discharge/pain, culture test results, antifungal sensitivity test results and associated features were documented in a proforma.

Inclusion criteria:

1. Presence of symptoms like itching, pain, ear discharge, feeling of a foreign body in the ear, hearing loss, or tinnitus.
2. Age 10-60 years
3. Culture positive cases

Exclusion criteria:

1. Patients above 60 years of age
2. Patients with suspected malignancies

Every recruited participant was examined with the help of otoscope and investigated for the presence of fungal agents through collection of 3 ear swabs from each patient. First swab was used for 10 % KOH mount, the second swab was used for 10 % Gram training and the third swab was used for 10 % inoculating the SDA media for 48-72 and incubated at 37C showing collection of sample with sterile cotton swab under all aseptic precautions. The media used to isolate the cultures included Sabouraud dextrose agar, Hicrome candida differential agar base (HIMEDIA), Blood agar, Mac conkeys agar.

Identification of specimen was conducted through: KOH mounts, SDA plates, Microscopic Culture Techniques and Lactophenol Cotton Blue preparation.

Anti-fungal susceptibility testing of the isolated specimens was performed using Disk Diffusion Testing with standard discs of anti-fungal agents such as Itraconazole, Posaconazole, Fluconazole and Amphotericin B as well as with a Candifast kit [10]. Candifast is a commercially available test kit that allows identification of pathogenic yeasts as well as testing of their resistance to various antifungals agents including Amphotericin B, Nystatin, Flucytosin, Econazole, Ketoconazole, Miconazole, Fluconazole.

The data obtained was spread in Microsoft excel sheets and analyzed using Microsoft Excel Windows 2010 version. Results were expressed as Mean ± SD. Data was analyzed with appropriate statistical methods.

Result and Observations:

Table No.1: Depicting Age-Wise Distribution:

Age group (years)	No.of cases	Percentage
10-20	182	19%
21-30	286	30%
31-40	259	27%

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41-50	105	11%
51-60	124	13%
Total	956	100%

Table 2: Depicting Sex-Wise Distribution:

Sex	No. of cases	Percentage
Male	431	45%
Female	525	55%

Graph 1: Depicting Laterality of Disease:

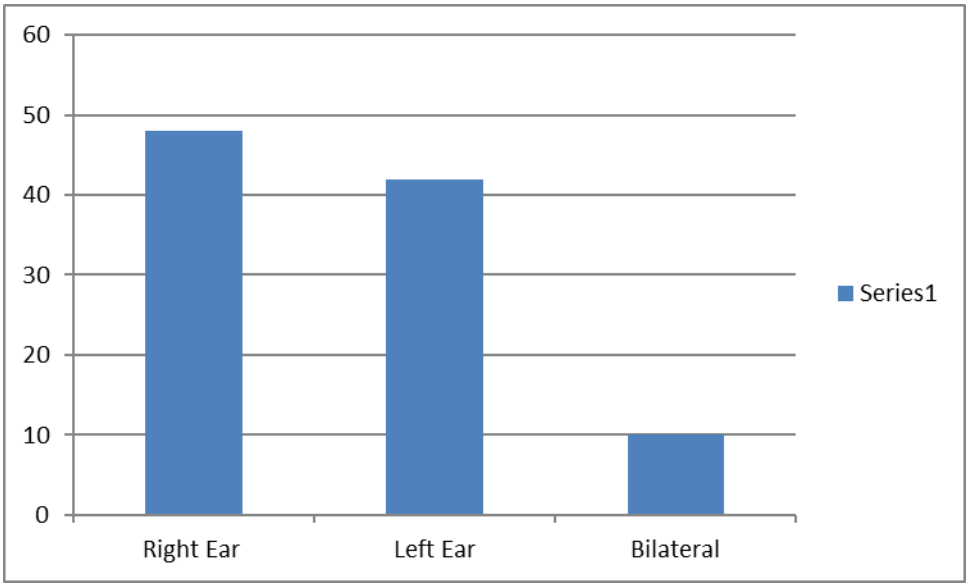

Table No. 3: Anti Fungal Agents Isolated:

FUNGAL AGENT	No.of cases	Percentage
Aspergillus flavus	143	15%
Aspergillus fumigatus	86	9%
Aspergillus niger	344	36%
Aspergillus terreus	9	1%
Candida albicans	106	11%

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Candida Globrata	10	1%
Candida Parapsilosis	19	2%
Candida Krusei	10	1%
Candida Tropicalis	19	2%
Penicillium notatum	10	1%
Fusarium	10	1%
No growth	191	20%

In our study, the swab material cultured in Sabouraud's dextorse agar showed that 143 cases were caused by Aspergillus flavus, 86 cases were caused by Aspergillus fumigatus, 344 cases were caused by Aspergilus niger, 106 cases were caused by Candida albicans, and 10 was Pencillium notatum cases. In total 582 cases were caused by aspergillus species and 164 cases caused by Candida albicans, and rest 10 was by Penicilium notatum.

Antifungal Sensitivity of the Isolated Specimen:

Table No. 4: Depicting Antifungal susceptibility testing by Disk Difussion Test:

Species	Sensitivity	AB(no. of cases/ percent)	ITRA(no. of cases/ percent)	POS(no. of cases/ percent)	VORI(no. of cases/ percent)
AspergillusFlavus	Sensitive	133(93.1%)	143(100%)	143(100%)	143(100%)
	Resistant	10(6.9%)	0(0%)	0(0%)	0(0%)
AspergillusFumigatus	Sensitive	86(100%)	86(100%)	86(100%)	86(100%)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)
Aspergillus Niger	Sensitive	344(100%)	324(94.2%)	324(94.2%)	324(94.2%)
	Resistant	0(0%)	20(5.8%)	20(5.8%)	20(5.8%)
Aspergillus terreus	Sensitive	9(100%)	9(100%)	9(100%)	9(100%)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)

Majority of the isolated entities were responsive to the routinely prescribed antifungal treatments.

In disk diffusion method for filamentous fungi. A.fumigatus & A.terreus were sensitive to all the antifungal in the plate. A.niger showed 5.8% resistances to itraconazole & A.flavus showed 6.9% resistances to ampho B.

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Table No. 5: Depicting Anti-fungal susceptibility results through Candi Fast Kit.

Species	Sensitivity	AB	NY	FCT	ECZ	KTZ	MCZ	FCZ
C.Albicans	Sensitive	106 (100%)	106 (100%)	106 (100%)	106 (100%)	106 (100%)	106 (100%)	86 (81.1%)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	20(18.8 %)
C.Glabrata	Sensitive	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)	10(52.6 %)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	9(47.3%)
C.Krusei	Sensitive	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
C.Tropicalis	Sensitive	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
C.Parapsilosis	Sensitive	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19 (100%)	19(100%)
	Resistant	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

In Candifast, C.tropicalis & C.parapsilosis were sensitive to all the antifungal in the kit. C.albicans & C.glabrata showed 18.8%,47.3% resistances to fluconazole.

DISCUSSION:

Otomycosis has been found to be a commonly diagnosed disease in India, its high incidence being attributed to the high degree of heat and humidity, dusty atmosphere as well as the fact that a major

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proportion of population here belong to outdoor laborers and low socio-economic class.[11,12]

In our study, we observed that females were more often affected with otomycosis, comprising 55% with a M:F ratio of 9:11; thereby, concluding that the sex of the individuals had a statistically significant relationship to the culture positivity. This result is in accordance with conclusions made by Mahmoud abadi and Zeba in 2010 [15]. However, these data are in disagreement with the findings by Yehia MM [16], Kour R [8], wherein greater percentage of the total subjects were found to be males.

Fungal Otitis media is widely known to be a disease of the young adults and in our research, 57 percent of the patients with culture positivity fell into the age group of 21-40. Similarly, studies conducted by Fasnula J [2] and more recently by Suharshi Gupta [11] found the most prevalent age group of otomycosis to be in the 3rd and 4th decade of life among the culture positive subjects of their study. While the lowest number of patients in the present study were found in the age group of 41-50, many studies found it the least number of patients to be below the age of 20. [2,11]

Almost all (90%) of the patients were diagnosed with unilateral ear involvement with 48 percent of them presenting with Right sided infection while the other 42% presented with Left sided infection. The remaining 10% of the subjects were found to have bilateral ear involvement. This finding is in accordance with the results of studies carried out by Paulose KO [17], Mugliston T [5] and more recently by Sampath Chandra Prasad [7]all of which have reported that otomycosis is predominantly a unilateral disease in 87%, 89% and 95% of their subjects respectively.

The current study encompassed only those patients who were culture positive upon examination. Though there were a variety of fungal species isolated, Aspergillus and Candida spp. were the common fugal species in our study, Aspergillus being the commonest one. Again, this finding is similar to many others from India as well as from other nations. [8, 13, 16].

Aspergillus species are found to be a common entity in airborne dust and the presence of wax in the ears facilitates its heavy growth. [5]. Moreover, since the disease-causing aspergilli experiences optimal growth in acidic environment (pH: 5-7), it exhibits heavy growth in the normal ear canal whose pH is on the acidic side.

In our study, A. niger (36%) was the most common specimen isolated, followed by A. Flavus (15%). This is in complete disagreement with the study conducted by others like Kaur R[8] in which A. flavus was found to infect only 1% of the study subjects with A. fumigatus being the most common etiological agent. This deviation from the results of other researches can be attributed to variations in the population groups, lifestyles, environmental conditions, as well as the procedure employed for isolation. Mixed fungal infection was found in none of our study population. Identification of the etiological agent is of paramount importance because different species have varying degree of susceptibility to the anti-fungal medications and it will dramatically affect the choice of anti-fungal agent subsequently. The prevalence of various fungi in percentages from varying studies is depicted in the Table no.5.

Anti-fungal susceptibility tests were performed for the isolated specimens deploying Disk diffusion method and Candifast Kit. The Disk diffusion method indicated that among the Aspergillus species, A. niger (the most common one) showed high susceptibility to Amphotericin B whereas it exhibited a 5.8 percent resistance to itraconazole, voriconazole and posiconazole. On the other hand, A.fumigatus and A. terreus showed 100 percent susceptibility to all four of the antifungal agents (table no. 3), wheras A. flavus demonstrated a 6.9% resistance to Amphotericin B.

The sensitivity testing of Candida Species displayed that Candida Albicans (the most common agent among Candida species) and C. glabarta were sensitive to all the antifungal agents (see table no. 4) except for fluconazole where they demonstrated 18.8% and 4.3% resistance respectively. This demonstration of resistance to fluconazole is similar to the results reported by Rawat Saritha and collegues [6]. However, C. krusei, C.parapsillosis and C. tropicalis were all completely sensitive to all the antifungal agents in the Kit which happens to vary from the results obtained in the study conducted by Rawat Saritha[6] in 2017.

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Table No. 5:

Comparison of prevalence (%) of various fungi in different studies of Otomycosis.					
Etiological Agent	Yehia et al, [16]1990	Kaur et al, [8] 2000	James Fasnula et al [2], 2007	JIa et al, China,[18] 2012	Rawat saritha,[6] 2017
A niger	70.9	36.9	48.35	54.77	58
A fumigatus	5.6	41.1	33.96	2.61	4
A flavus	15.6	1.4	5.43	6.09	23
Other Aspergillus	0	0	0	9.57	0
Candida species	7.3	13.7	12.26	24.35	12
Mucor species	0	1.4	0	0	0
Penicillium species	0	1.4	0	0	1
Rhizopus species	0.6	2.7	0	0	0
Other fungi	0	1.4	0	2.61	2

These differences in the results explicitly reflects that each case of otomycosis should not be treated merely on Out-patient department basis, but shall rather be sent for fungal culture testing along with antifungal drug susceptibility testing for prescribing the most effective treatment regimen.

CONCLUSION:

The predominant etiological agent of Otomycosis in the present study was found to be Aspergillus Niger followed by A. flavus, A.fumigatus and Candida albicans.It can be inferred that A. flavus has become more common pathogen than A.fumigatus in the otomycosis compared to the previous experiments. (see table no. 5) Antifungal susceptibility testing results demonstrate high sensitivity to Amphotericn B by A.niger, A.fumigatus, A.terreus but a 6.9% resistance by A.flavus. The predominant candida species (c. albicans) exhibits susceptibility to all the drugs except for fluconazole where it shows an 18.8 percent resistance. Therefore, anti-fungal susceptibility testing along with culture tests shall be performed prior to prescribing patient regimen since they are instrumental in deciding the most effective treatment plan. Patients with helicobacter pylori infection.

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