

Clinicomycological Study of Otomycosis with Antifungal Susceptibility Testing Of Fungal Isolates.

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Abstract

Otomycosis or fungal otitis externa is a superficial, sub-acute or chronic infection of the external auditory canal, usually with unilateral presentation characterized by inflammation, pruritis, scaling and otalgia.

Aim Of The Study:

To find the prevalence of otomycosis along with its clinicomycological profile, to explore specific predisposing factors, to isolate and identify the causative fungus and antifungal susceptibility testing for moulds by E test and for yeast by disk diffusion method.

Methodology: Ear swabs were collected from 30 clinically suspected cases of otomycosis and all the samples were processed by direct microscopy of KOH mount and Gram staining. Culture was carried out on SDA. The identification process of the isolated fungus was done by standard procedures. Antifungal sensitivity of moulds was performed on RPMI 1640 agar supplemented with 2% glucose and tested for fluconazole (0.016-256 mcg/ml), Itraconazole (0.002 mcg/ml to 32 mcg/ml) and Caspofungin (0.002-32 mcg/ml) (HiMedia) by E strip. AFST for yeast was performed by the disc diffusion method on Mueller Hinton Agar supplemented with 2% glucose and 0.5mg/ L Methylene Blue (HiMedia) and tested for Fluconazole (10µg), Nystatin (100U), Amphotericin (100U), Ketoconazole (10µg) and Clotrimazole (10µg) disk (HiMedia).

Result And Conclusion: Otomycosis was diagnosed in 100% of the cases with highest prevalence in 21-40 years of age group with a female preponderance. Itching was found to be the commonest symptom followed by ear pain. Ear pricking with hard objects was found to be the most common predisposing factor followed by swimming or pond bath. Seven cases were associated with tympanic membrane perforation. Of the 30 cases 32 number of fungi were isolated. Fungal isolates were predominantly mould (90.62%) while rest were yeast (9.38%). Mixed infection with more than one fungus was seen in 2 cases (6.67%). *A.niger* was the predominant fungus (71.88%) followed by *A.flavus* (18.75%) and *C.tropicalis* (9.37%). All the yeasts were found to be sensitive to Ketoconazole, Nystatin and Amphotericin and all moulds were found to be resistant to fluconazole.

Keywords: Otomycosis, *Aspergillus*, Antifungal susceptibility testing.

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I. Introduction

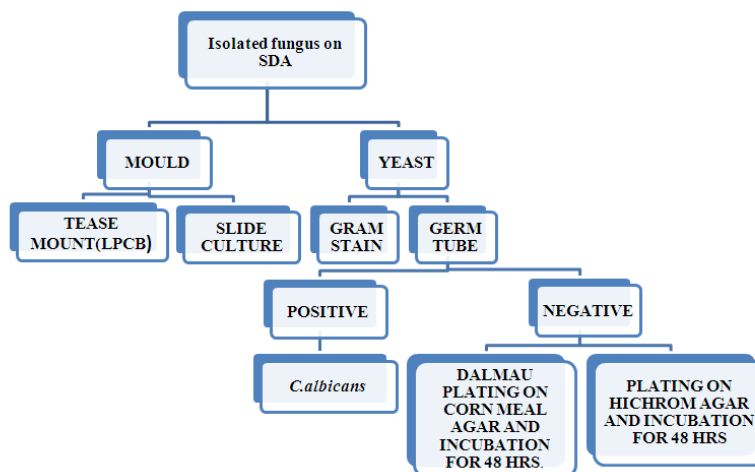
Otomycosis or fungal otitis externa is a superficial, sub-acute or chronic infection of the external auditory canal, usually unilateral, which is characterized by inflammation, pruritis, scaling and otalgia⁽¹⁾. It is more prevalent in warm and humid climates, particularly in monsoon season⁽²⁾. Prevalence is also influenced by the geographical area, as otomycosis is most commonly present in tropical and subtropical humid warm climates⁽³⁾. Factors that predispose to fungal otitis externa include absence of cerumen, high humidity, increased temperature, bacterial otitis externa, corticoid therapy, swimming⁽⁴⁾ and local trauma – caused by sharp objects like sticks or hearing aids. Contributing factors for otomycosis included use of oils and eardrops, use of wooden sticks, feather and metal wax picks and history of diabetes. Otomycosis is found in all age groups, but majority of the cases of otomycosis occur in patients aged 21 -30 years with equal male female distribution⁽⁶⁾. *Aspergillus* and *Candida spp.* are the most frequently isolated fungi in patients with otomycosis⁽⁷⁾.

Present study has been undertaken to determine the prevalent causative agents of otomycosis and know their antifungal susceptibility pattern in patients attending ENT Department at M.K.C.G Medical College and Hospital, Berhampur.

II. Materials And Methods

The study involved 30 patients who presented with symptoms of otomycosis at ENT OPD of M.K.C.G MCH, Berhampur from August 2018 to September 2018.

Clinical details and predisposing risk factors were recorded. Clinical samples collected with two sterile swabs. One swab was used for microscopy by KOH(10%) mount and gram stain. The second was inoculated onto two SDA tubes and incubated. The identification process of the isolated fungus was done by growth characters on SDA, cornmeal agar, Hichrom agar and microscopic appearance on LPCB tease mount.



ANTIFUNGAL SUSCEPTIBILITY TESTING

A) FOR MOULD-

Fungus was cultured on potato dextrose agar for 5-7 days to induce conidia of filamentous fungi. Five millilitres of 0.85% normal saline, 10µl of tween 80 and a loopful growth of test organism were taken in a test tube. Optical densities of conidial suspensions were adjusted and read at 530 nm. OD value ranges from 0.09-0.13 for *Aspergillus spp.*

For moulds Inoculum was lawn cultured on RPMI 1640 supplemented with 1.5 % agar and 2% glucose buffered to a pH of 7.0. and tested for fluconazole (0.016-256 mcg/ml), Itraconazole(0.002 mcg/ml to 32 mcg/ml) and Caspofungin (0.002-32 mcg/ml) E strips and incubated at 35°C for 24 hrs .

B) FOR YEAST-

Twenty four hours growth from Sabouraud dextrose agar at 35°C was taken for inoculum preparation. Normal saline(0.85%) and 5 colonies from the above were taken in a test tube. Optical density of inoculum was tested against 0.5 McFarland.

For yeasts disc diffusion method was performed on Mueller Hinton Agar supplemented with 2% glucose and 0.5mg/ L Methylene Blue and tested for Fluconazole(10µg), Nystatin(100U), Amphotericin (100U), Ketoconazole(10µg), Clotrimazole(10µg) disk incubated at 35°C for 24 hrs.

III. Results

TABLE 1: DISTRIBUTION OF CASES ACCORDING TO THE AGE GROUP AND GENDER.

AGE IN YEARS	MALE N=13 (43.33%)	%AGE	FEMALE N=17 (56.67%)	%AGE
0-10	1	7.69	3	17.65
11-20	3	23.08	0	0
21-30	3	23.08	5	29.41
31-40	3	23.08	5	29.41
41-50	2	15.38	3	17.65
51-60	1	7.69	1	5.88
>60	0	0	0	0

TABLE 2: PREDISPOSING FACTORS

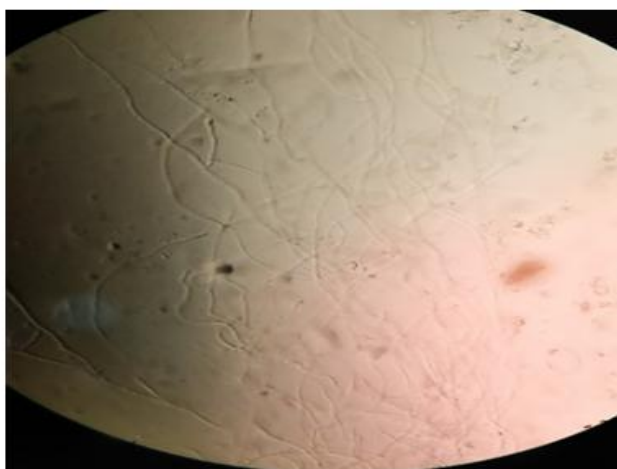
PREDISPOSING FACTORS	NUMBER OF CASES	% AGE WHEN TOTAL NUMBER OF CASES (N=30)
SWIMMING OR POND BATH	8	26.67
USE OF OIL	6	20
DIABETES OR OTHER IMMUNODEFICIENCY DISEASES	5	16.67
EAR PRICKING WITH HARD OBJECTS	10	33.33
NO PREDISPOSING FACTORS	6	20

TABLE 3: SYMPTOMS

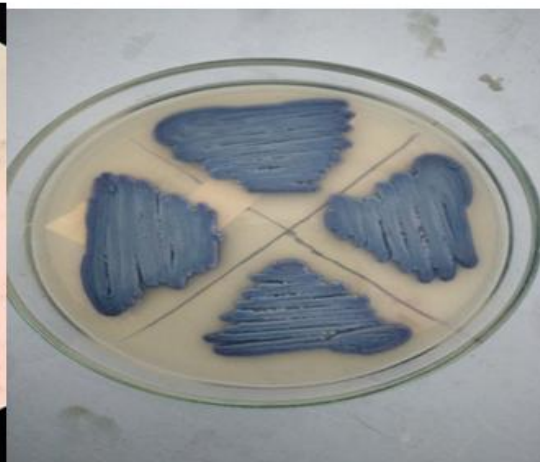
SYMPTOMS	CASES	% AGE When N=30
ITCHING	24	80
DISCHARGE	5	16.66
EAR PAIN	18	60
SENSATION OF EAR BLOCKAGE	7	23.33
DECREASED HEARING	6	20

TABLE 4: FUNGI ISOLATED

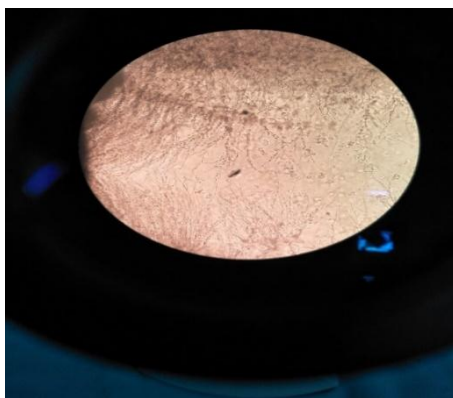
	FUNGAL ISOLATE	CASES	% AGE
1	Only <i>A.niger</i>	21	70
2	Only <i>A.flavus</i>	4	13.33
3	Only <i>C.tropicalis</i>	3	10
4	Mixed infection	2	6.67



KOH MOUNT(10%) SHOWING FUNGAL ELEMENTS



HICHROM AGAR SHOWING GROWTH OF *C.tropicalis*



C.tropicalis in DALMAU PLATING

ANTIFUNGAL SUSCEPTIBILITY TESTING RESULTS

TABLE 5: OBSERVED MIC RANGE OF MOULDS

FUNGAL ISOLATE	OBSERVED MIC RANGE OF FUNGAL ISOLATES IN µg/ml		
	ITRACONAZOLE	CASPOFUNGIN	FLUCONAZOLE
<i>A.niger</i>	0.006-1.0	0.125-1.0	0
<i>A.flavus</i>	0.006-0.064	0.125-0.75	0

All moulds were found to be resistant to fluconazole

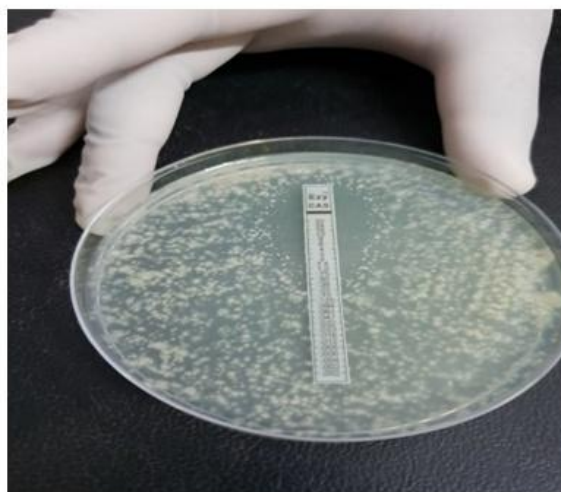
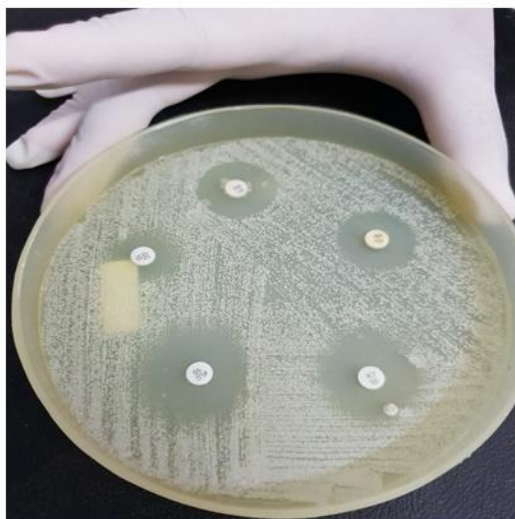


TABLE 6: SENSITIVITY PATTERN OF YEASTS

ANTIFUNGALS	NO.OF ISOLATES SENSITIVE	% AGE	NO.OF ISOLATES RESISTANT	% AGE
Ketokonazole (10 µg/ml)	3	100	0	0
Fluconazole (10µg/ml)	1	33.33	2	66.67
Cotrimazole (10µg/ml)	1	33.33	2	66.67
Nystatin (100U)	3	100	0	0
Amphotericin (100U)	3	100	0	0



All the yeasts were found to be sensitive to Ketoconazole , Nystatin and Amphotericin

IV. Discussion

Otomycosis is a fungal ear infection, a superficial mycotic infection of the outer ear canal which is more prevalent in tropical countries. It may present as acute or subacute infection. The mycosis results in inflammation causing superficial epithelial exfoliation, accumulation of debris containing fungal hyphae, pain and suppuration⁽⁸⁾. The commonest finding on ear examination is the presence of greyish white thick debris and heaviness in the ear.

In our study highest prevalence was seen in the age group of 21-40 years (53%) which is comparable with other studies showing higher incidence among 21-30 years of age^(8,10,11,12-15). This may be because people of this age group are the active age group involved in different activities which expose them to the infectious agent present in the air.

Female preponderance is seen in our study (56%) which is similar to the other studies^(8,16,17) while studies conducted by Satish HS *et al.*,⁽¹⁰⁾ and Prasad SC *et al.*,⁽¹¹⁾ has shown male preponderance. Female while doing house hold work are exposed to dusty air containing spores making them vulnerable to the fungal infection.

Ear pricking with hard objects was the commonest predisposing factor (33%) followed by pond bath, use of oil and diabetes. Studies conducted by Rawat Sarita *et al.*,⁽⁹⁾ have shown history of using wicks was present in 35%, followed by association with diabetes in 7% and swimming history in 4% cases. Use of hard objects to clean the ear canal leads to trauma in which fungal spores settle and cause infection. Swimming in ponds and rivers is a common way of getting such infection because water trapped in ear canal after bathing in a humid climate can cause fungal infection of the ear⁽¹⁸⁾ and use of oil act as a risk factor because fatty acids in oil gives a suitable medium for the growth of the fungus.

The commonest presenting symptom was itching in 80% of cases followed by ear pain (60%) which is similar to findings by other researchers⁽⁹⁾. Inflammation of the ear canal leads to itching and pain and other symptoms like sensation of ear blockage, discharge and decreased hearing may be due to accumulation of fungal debris in the ear canal.

Among the 30 cases, fungi were isolated from all 30 cases (100%) with 32 isolates whereas other studies revealed growth of fungi from 94% cases⁽⁹⁾ and 51.3% cases⁽⁸⁾. This higher incidence of fungal growth can be attributed to hot and humid climate of the southern part of Odisha.

Most of the fungal ear infections are caused by members of *Aspergillus spp* like *A.niger*, *A.fumigatus* and *Candida albicans*⁽⁹⁾ which is seen in our study having *A.niger* as the predominant pathogen in 77% of cases.

In our study the observed range of MIC in *A.niger* for Itraconazole was 0.006-1.0 µg/ml and Caspofungin was 0.125-1.0 µg/ml and in *A.flavus* for Itraconazole was 0.006-0.064 µg/ml and for Caspofungin was 0.125-0.75 µg/ml. All moulds were found to be resistant to fluconazole and all the yeasts were found to be sensitive to Ketoconazole, Nystatin and Amphotericin.

Antifungal resistance is on the rise, and is an emerging threat to patient management and clinical success. The global spread of azole-resistant *Aspergillus* species is particularly concerning. Triazoles are recommended for the treatment of aspergillosis, and are widely used for the treatment of candidiasis⁽¹⁹⁾. Epidemiological studies report substantial azole resistance among *Candida* and *Aspergillus* species^(20,21). Azole resistance among *Aspergillus* species reflects an increase in drug use for prophylactic and long-term treatment

regimens, and acquired azole resistance was reported in patients who had undergone long-term treatment with azoles⁽²¹⁾.

V. Conclusion

Otomycosis has worldwide distribution but is more prevalent in tropics and subtropics due to hot and humid climatic conditions. Human external auditory canal is an ideal environment for these fungi to grow. Fungal infection of external auditory canal should be suspected in all chronic cases of otitis externa which do not respond to conventional topical therapy. *Aspergillus* spp was the commonest etiological agent isolated which showed resistance to common antifungals, susceptibility testing can assist with determining the appropriate drug treatment and respective epidemiology. E test is useful, readily available and easy to use test in determining in vitro susceptibility of *Aspergillus* spp.

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