Original Research Article

A study on changing trends of antifungal susceptibility in isolates from cases of otomycosis

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A R T I C L E  I N F O

Article history:
Received 25-01-2021
Accepted 26-02-2021
Available online 26-03-2021

Keywords:
Otomycosis
Antifungal Candida Albicans
Antifungal susceptibility

A B S T R A C T

Background: Otomycosis may be refractory to the treatment prescribed and challenges the clinicians to determine whether the external ear disease is an isolated entity or related to any other systemic disorder or the result of underlying immune deficiency disorder.

Aim & Objective: The aim of the study was to identify the causative organism associated with otomycosis with special reference to aspergillus and candida species. On the basis of finding of this study, treatment of otomycosis due to aspergillus/candida species can be better when a particular species is isolated and its antifungal sensitivity pattern is known.

Materials and Methods: The present study “CHANGING TRENDS OF ANTIFUNGAL SUSCEPTIBILITY IN ISOLATES FROM CASES OF OTOMYCOSIS” was undertaken in the Department of microbiology at Ayaan Institute of Medical Sciences, Hyderabad & KIMS Narketpally. The study was conducted over a period of 1 year from April 2019 to April 2020. The study group comprised of 100 patients presenting with complaints of itching and ear ache to the ENT outpatient department at Ayaan Institute of Medical Sciences & KIMS. Patients were evaluated as per the study protocol ear swabs were collected and processed as per the standard microbiological techniques for the isolation and identification of the organism. Different etiological agents were identified with special reference to aspergillus and candida species.

Results: In the present study maximum number of patients was in the age group of 21-30 years (30%) from low socio-economic status presenting with itching, ear ache and decrease hearing. Prevalence of otomycosis was high in females mostly due to injury to the ear canal with hairpin followed by use of antibiotic ear drops. Out of 100 study group, 61% had fungal infection by aspergillus species followed by candida species 17% and normal flora or no growth was seen in 24% cases. Among the aspergillus species 4 species were identified. A.Niger(36%), A.Flavus(15%), A.Fumigatus(9%), A.terreus(1%) and in candida species 5 species were identified. Antifungal susceptibility testing of aspergillus isolates by disk diffusion revealed that A.Fumigatus, A.terreus were sensitive to all the 4 drugs listed. Aspergillus niger showed 94.4% sensitivity to triazole drug followed by aspergillus flavus which showed 93.3% sensitivity to polyene. Antifungal susceptibility candida isolates by candifast revealed that C.cruze, C.Tropicalis, C.Paracilosis were sensitive to all the seven drugs in the kit(AB;NY;FCT;ECZ;KTZ;MCZ;FCZ) C.Albicuns showed 81.9% sensitivity to triazole drug followed by C.glabrata which showed 50% sensitivity.

Conclusion: Finally we concluded that, aspergillus species were most commonly isolated in patients presenting itching, ear ache and hearing loss, the most predominant being A.niger followed by A.flavus then A.Fumigatus also there were other like candida albicans, penicillium. Few cases showed normal ear flora. Antifungal susceptibility pattern of the study by disk diffusion revealed that A.Fumigatus, A.terreus were sensitive to the entire drug in the disk. Subsequent follow up of the patient receiving antifungal drug for almost 2 weeks showed marked resolution of symptoms.

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1. Introduction

Otomycosis or fungal otitis externa is defined as a fungal infection of the external auditory canal. It is a superficial subacute or chronic fungal infection of the external ear canal; but may extend to the middle ear with a perforated tympanic membrane. Otomycosis can also be associated with chronic middle ear disease and open mastoid cavity infection.

It is a common complaint at the ENT outpatient clinic with prevalence ranging from 9% in temperate climates to as high as 54% in hot and humid regions. It is worldwide in distribution with most fungi responsible for infection being saprophytic. Rarely are established pathogens recovered from these patients. The candida species pencicullium, mucor and rhizopus species, have also been implicated. 1,2

Otomycosis may be refractory to the treatment prescribed and challenges the clinician to determine whether the external ear disease is an isolated entity or related to any other systemic disorder or the result of underlying immune deficiency disorder. Fungi can be either primary pathogens or secondary invaders of tissue left vulnerable following a previous bacterial infection, eczema, physical injury or accumulation of cerumen. It is more common in an immune compromised host with incidence increasing with the widespread and prolonged use of broad spectrum antibiotics, steroids and chemotherapeutic agents. 3

Most of the otomycosis cases are therapeutic failures referred from general practitioners, underlying repeatedly the frequency and morbidity of the condition. The management is dependent on thorough understanding of anatomy and physiology of the external ear canal, knowledge of microbiology of potential pathogens and familiarity with clinical presentation, so that an accurate and timely diagnosis can be reached. 4

Control of infection can be obtained by frequent cleaning of fungal debris from the external canal so that topical therapy can be effective. It is worth stressing that there are no reports on the toxicity of antifungal drugs in literature. There is increasing evidence from recent studies of an increasing resistance to Fluconazole, Itraconazole, Amphotericin B, Ketoconazole and Nystatin among the common pathogenic species. 5

This study was undertaken to identify fungal species involved in otomycosis, their distribution and predisposing factors and their sensitivity to antifungal therapeutic agents in patients living in and around the area of Ayaan Institute of Medical Sciences, Telangana State.

2. Aims & Objectives

The main objectives of this research “Microbiological study of otomycosis with changing antifungal susceptibility and resistance” are

1. To study the spectrum of fungal etiological agents among patients clinically diagnosed as having otomycosis in the ENT OPD of AIMS & KIMS Hyderabad.
2. To study the epidemiological pattern of fungi infecting the ear.
3. To perform antifungal susceptibility testing of isolates and to assess the changing trends in sensitivity patterns.
4. To determine the use of antifungals in the treatment of otomycosis in patients attending ENT OPD of AIMS & KIMS.

3. Materials and Methods

A Study group comprising of 100 cases, presently with complaints or itchy earache were selected from patients attending. The ENT outpatient department of AIMS & KIMS Hyderabad during a period one year from 2019 April to 2020 April.

A detailed history regarding Age / Occupation Social class, underlying disease Trauma was taken in a proforma. Duration or the complaints, nature of the discharge and associated feature were included as well in the proforma.

3.1. Inclusion criteria

1. Common presently symptoms like Itching, Pain, Ear discharge, feeling or a foreign body in the ear hearing loss or tinnitus were considered. All case hairy the above symptoms sign suggestive of otomycosis like, cotton wooly mass, a wet newspaper like debris in ext. Auditory canal were taken in the study.
2. The age group or pt included in this study was from 10 to 60 years.
3. Only then which were culture positive were taken for the study.
4. Patient above 60 years were excluded
5. Patient with suspected malignancies

3.2. Exclusion criteria

1. The age group or pt included in this study was from 10 to 60 years.
2. Only then which were culture positive were taken for the study.

3.3. Methodology

3.4. Collection of sample

Patients attending the ENT outpatient department with specific complaints of Itching, Ears discharge were examined with the help of otoscope. Nature of debris and condition of ext. auditory and canal was noted.

3 Ear swabs were collected and processed immediately

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1. First swab was used for 10% KOH mount
2. Second swab was used for 10% Gram stain
3. Third swab was used for 10% inoculating the SDA media for 48-72 & incubated at 37°C

4. Procedure for performing the disk diffusion test

   Inoculum Preparation: Direct colony suspension method

4.1. Investigation

4.1.1. Hyline molds

   Wet preparations - two swabs are taken one for KOH mount and another for CULTURE

   4.1.1.1. KOH Mounts. Slide KOH – place skin scraping, debris on a clean glass slide. Pour drop of 10% KOH on specimen and place coverslip over it. Heat the slide gently over flame and examine under microscope after a few minutes.

   4.1.1.2. Culture on SDA plate. It is a selective medium for the isolation of pathogenic fungi from clinical specimens by inhibiting the growth of bacterial.

   The sample was inoculated on a SDA and incubated at 35°C. Culture were examined within 2 days for appearance of growth. Culture were incubated for 1 week and discarded if no growth occur. In this the spore structures and arrangements are seen.

   4.1.1.3. Microscopic culture technique:

     1. Place around piece of filter paper on bottom of a petri dish. Place a pair of thin glass rods on the top of filter paper to serve as supports for a 3x1 inch in glass microscopic slide

     2. Place a block of SDA on the surface of the microscope slide.

     3. Inoculate the margins of the agar plug in three or four places with a small portion of the colony to be studied, using a straight inoculating wire. Gently heat cover slip by passing it quickly through the flame of bunsen burner and place it on the surface of the inoculated agar block.

     4. Pipette small amount of water into bottom of petri dish to saturate the filter paper. Incubate at 37°C for 3 to 5 days

     5. Place the cover slip on small drop of PCB mount. This should be performed under a biological safety hood. Now the agar block can be removed. This is stained with PCB and a cover slip is overlaid.

   4.1.1.4. Lactophenol cotton blue. A drop of LPCB was placed on the centre of a clean glass slide using a sterile needle a small portion of fungal colony was transferred to the drop of LCB on the slide, emulsified & cover slip was placed & examined under; low power.

4.2. In-vitro susceptibility testing in aspergillus species

4.2.1. DISK DIFFUSION METHOD [CLSI M44-A2]

   Agar medium: MHA+2% dextrose & 0.5ug of methylene blue dye/ml. Inoculum preparation: from 24hrs culture on SDA. Test medium: stock inoculum suspension, adjusted by spectrophotometer at 530 nm to match the turbidity of a 0.5 me farland standard1*106 to 5*106 cfu/ml. Disk contents: fluconazole 25u, itroconazole 10u, Poseonazole 5u, voriconazole lug, Incubation: 20-24 h at 35c

   Mean of End Point Determination.

   | AFD   | S     | SD D | R     |
   |-------|-------|------|-------|
   | FLU   | >19 MM| 15- 18 MM | <14 MM |
   | VORI  | >17 MM| 14- 16 MM | <13 MM |

4.2.2. Agar based methods

   Etest (commercial method)- it is easy to perform, contamination can be recognized and in vitro resistance may be distinguished.

   Method - directly quantifies antifungal susceptibility in terms of discrete MIC values. RPMI based agars more useful.

   Interpretations showed good correlation with amphoB, itroconazole for aspergillus spp. detecting caspofungin resistance in Afumigatus.

   E test is a good alternative methods for determine the antifungal activity of is a vaconazole again aspergillus.

4.3. Observation and Results

   One hundred cases of otomycosis attending ENT outpatient department AIMS & KIMS, were studied in the Department of observation were made as follows:

   Total no. of cases 100

   | Table 1: Depicting age wise distribution |
   |----------------------------------------|
   | Age group (years) | No. of cases | Percentage |
   |-------------------|--------------|------------|
   | 10-20             | 19           | 19%        |
   | 21-30             | 30           | 30%        |
   | 31-40             | 27           | 27%        |
   | 41-50             | 11           | 11%        |
   | 51-60             | 13           | 13%        |
   | Total             | 100          | 100%       |

   In this study patient of ages from 10-60 years was taken into consideration. The youngest was 10 years female patient and eldest patient was 60 year old male.

   Maximum numbers of cases were between 21-30 years of age (60%).

   Out of one hundred cases 45% were male and 55% were female.

   In this study, the incidence of otomycosis was more among females.
Table 2: Depicting sex distribution

| Sex    | No. of cases | Percentage |
|--------|--------------|------------|
| Male   | 45           | 45%        |
| Female | 55           | 55%        |

Table 3: Depicting distribution of otomycosis subject according to the side of ear affected

| Side affected | No. of cases | Percentage |
|---------------|--------------|------------|
| Right Ear     | 48           | 48%        |
| Left Ear      | 42           | 42%        |
| Bilateral     | 10           | 10%        |

In this study the incidence of otomyosis with respect to laterality was 90% unilateral, 10% bilateral of the 90% unilateral disease 48% were of right side and 42% left side.

Table 4: Depicting occupation wise incidence

| Occupation | No. of cases | Percentage |
|------------|--------------|------------|
| Business   | 18           | 18%        |
| Employee   | 8            | 8%         |
| Farmer     | 22           | 22%        |
| House wives| 45           | 45%        |
| Student    | 7            | 7%         |

Out of 100 cases 45 were housewives, 8 were employees, 22 were farmers, 7 were students, 18 were businessmen.

In our study the incidence of otomycosis was high among housewives, and least among farmers. The comparative outpatient strength of housewives is higher when compared to other groups.

Table 5: Depicting area wise distribution

| Locality | No of Patients | Percentage |
|----------|----------------|------------|
| Rural    | 86             | 86%        |
| Urban    | 14             | 14%        |

Out of 100 cases 86 were Rural, 14 were Urban. About table shows the incidence of otomycosis was common in Rural area compared to the urban area because of poor hygienic and unhealthy living conditions.

The above study was conducted from April 2019 to April 2020 which includes duration of 12 months. In this study majority of cases were obtained during May to December this period in Telangana, Hyderabad includes summer and rainy season.

In this study the predominant complaint of the patient was itching 50% and the least common complaint was ear discharge. 22% had all complaints. Isolated presentation of itching was seen in 10% of cases. Combination of itching and pain was found in 18% of cases.

In our study the most common pre-disposing factor was injury to canal (31%) and use of ear drops (43%).

In the above study there was no presence of wax in the external auditory canal with otomyosis.

Table 6: Depicting season wise incidence

| Month   | No. of cases | Percentage |
|---------|--------------|------------|
| June    | 20           | 20%        |
| July    | 21           | 21%        |
| August  | 18           | 18%        |
| September | 8         | 8%         |
| October | 6            | 5%         |
| November | 4          | 4%         |
| December | 3           | 3%         |
| January | 3            | 3%         |
| February | 4           | 4%         |
| March   | 2            | 2%         |
| April   | 3            | 3%         |
| May     | 8            | 8%         |

Table 7: Depicting presenting complaints

| Symptoms           | No. of cases | Percentage |
|--------------------|--------------|------------|
| Itching            | 50           | 50%        |
| Pain               | 22           | 22%        |
| Ear discharge      | 10           | 10%        |
| Hearing loss       | 18           | 18%        |

Table 8: Depicting pre-disposing factors

| Factors                      | No. of cases | Percentage |
|------------------------------|--------------|------------|
| Swimming/water entry into ear canal | 18 | 18% |
| Use of ear drops             |              |            |
| a) Use of antibiotics drops  | 9            | 9%         |
| b) Antibiotic and steroid drops | 6       | 6%         |
| c) Use of coconut oil        | 4            | 4%         |
| d) Wax solvent               | 2            | 2%         |
| e) None                      | 10           | 10%        |
| Injury to canal wall         |              |            |
| a) Broom stick               | 7            | 7%         |
| b) Cotton bud                | 5            | 5%         |
| c) Hairpin                   | 10           | 10%        |
| d) Match stick               | 9            | 9%         |
| e) None                      | 12           | 12%        |
| Associated systemic disease  |              |            |
| a) Diabetes                  | 4            | 4%         |
| b) HIV                       | 1            | 1%         |
| c) None                      | 3            | 3%         |

Table 9: Depicting role of wax

| Wax | No. of cases | Percentage |
|-----|--------------|------------|
| Present | 0            | 0%         |
| Absent | 100          | 100%       |

Table 10: Depicting otoscopic findings

| Findings                  | No. of cases | Percentage |
|---------------------------|--------------|------------|
| Black mycological plug    | 46           | 46%        |
| Cotton wooly mass         | 10           | 10%        |
| Dry mycelial matt         | 14           | 14%        |
| Soft debris               | 13           | 13%        |
| Wet mycelial matt debris  | 17           | 17%        |
In our study the otoscopic findings revealed black mycological plug in the external auditory canal which was the commonest presentation (46%), followed by wet mycelial matebris (17%), dry mycelial mat (14%) and soft debris (13%). Presentation in the form of cotton wooly mass was in 10% of cases.

| Table 11: Depicting associated ear disease |
|-------------------------------------------------|
| **No. of Cases** | **Percentage** |
| CSOM                      | 16  | 16%  |
| Congested tympanic Membrane | 12  | 12%  |
| Congested tympanic Membrane with oedema of Canal | 41  | 41%  |
| Mastoid Cavity             | 4   | 4%   |
| None                       | 24  | 24%  |

This is study otomycosis was associated with ear disease. Most commonly 41% had Congested tympanic membrane with oedema of Canal, 16% had CSOM, 12% has Congested tympanic membrane, 4% had otomycosis in post mastoid and there was no associated disease in 24%.

| Table 12: Depicting fungal species |
|-------------------------------------------------|
| **No. of cases** | **Percentage** |
| Aspergillus flavus                      | 15  | 15%  |
| Aspergillus fumigatus                  | 9   | 9%   |
| Aspergillus niger                      | 36  | 36%  |
| Aspergillus terreus                    | 1   | 1%   |
| Candida albicans                       | 11  | 11%  |
| Candida globrata                       | 1   | 1%   |
| Candida parapsilosis                   | 2   | 2%   |
| Candida krusei                         | 1   | 1%   |
| Candida tropicalis                     | 2   | 2%   |
| Penicillium notatum                    | 1   | 1%   |
| Fusarium                               | 1   | 1%   |
| No growth                              | 24  | 24%  |

In our study, the swab material cultured in sabouraud’s dextorse agar showed that 15 cases were caused by aspergillus flavus, 9 cases were caused by aspergillus fumigatus, 36 cases were caused by aspergillus niger, 11 cases were caused by candida albicans, and 1 was pencillium notatun cases. In total 62 cases were caused by aspergillus species and 11 cases caused by candida albicans, and rest 1 was by penicilium notatum.

Most of the isolates were sensitive to the routinely used antifungal.

In disk diffusion method for filamentous fungi A.fumigatus & A.terreus were sensitive to all the antifungal in the plate. A.niger showed 5.5% resistances to itraconazole & A.flavus showed 6.6% resistances to ampho B.

In candifast C.tropicalis & C.parapsilosis were sensitive to all the antifungal in the kit. C.albicans & C.glabrata showed 18.1%, 50% resistances to fluconazole.

5. Discussion

Chronic infective disorders of the ear remain a common source of misery for patients and frustration for clinicians. Otomycosis remains an uncommon disease often overlooked and goes unnoticed, unattended until severe pain occurs. Several authors have drawn attention to various aspects of otomycosis.

Fungi could be mentioned as a normal micro-flora in the external auditory canal playing an important role in otomycosis. Various factors pave the way for such saprophytic organisms to gain a foothold in the external canal. Fungal infections in the ear are increasing especially with indiscriminate use of topical antibiotic-steroid preparations and unhygienic habits. Treatment of otomycosis consists of adequate aural toilet, altering the pH of the external canal with acidifying agents and anti-fungal topical ear drops.

5.1. Age

The incidence of otomycosis was found to occur in age groups 10-60 years. The youngest case in this study was a 10 year old female who had otomycosis. The oldest patient in the study was a 60 year old male who was a diabetic. The study reveals that fungal infection was more common among young and middle aged individuals. The group from 21-30 years constitutes 56% of incidence. The above age group 11-30 years includes predominantly housewives and students. Housewives are exposed to cold damp environment doing household chores inside as well as in the fields and students are exposed to outside environment. The age incidence is in accordance with the studies of T.Mugliston and G.O’Donoghue (1985) 21%, Yehia MM and Al Habib HM and Shehab NM (1990) 48%.

5.2. Sex

In this study the incidence of otomycosis was found more among females (55%). This is in accordance with the study conducted by Yehia MM, and Al Habib HM, and NM Shehab (1990). The young and middle aged females are among the most common to be affected by the disease. Housewives in India as a whole and in South India in particular have a lot of family burden to share as predominantly the males work in fields, working in damp, cold conditions at house and field’s lead to exposure to dust and deposition of fungal spores. The unhygienic practice of self cleaning of the ear canal with dirty fingers, hair pins, match sticks hastens the deeper invasion of the fungus. However T.Mugliston and G.O’Doughue (1989) from London in their study found little difference in the sex incidence.
Table 13: Depicting Antifungal susceptibility testing by disk diffusion

| Species            | Sensitivity | AB  | ITRA | POS  | VORI |
|--------------------|-------------|-----|------|------|------|
| Aspergillus Flavus | Sensitive   | 14(93.3%) | 15(100%) | 15(100%) | 15(100%) |
|                    | Resistant   | 1(6.6%) | 0(0%) | 0(0%) | 0(0%) |
| Aspergillus Fumigatus | Sensitive   | 9(100%) | 9(100%) | 9(100%) | 9(100%) |
|                    | Resistant   | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| Aspergillus Niger  | Sensitive   | 36(100%) | 34(94.4%) | 34(94.4%) | 34(94.4%) |
|                    | Resistant   | 0(0%) | 2(5.5%) | 2(5.5%) | 2(5.5%) |
| Aspergillus terreus| Sensitive   | 1(100%) | 1(100%) | 1(100%) | 1(100%) |
|                    | Resistant   | 0(0%) | 0(0%) | 0(0%) | 0(0%) |

Table 14: Depicting Antifungal susceptibility testing by Candifast

| Antifungal Testing by Candifast | Species | Sensitivity | AB  | NY  | FCT | ECZ | KTZ | MCZ | FCZ |
|---------------------------------|---------|-------------|-----|-----|-----|-----|-----|-----|-----|
| C.albicans                      | Sensitive | 11(100%) | 11(100%) | 11(100%) | 11(100%) | 11(100%) | 11(100%) | 9(81.9%) |
|                                 | Resistant | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 2(18.1%) |
| C.glabrata                      | Sensitive | 2(100%) | 2(100%) | 2(100%) | 2(100%) | 2(100%) | 2(100%) | 1(50%) |
|                                 | Resistant | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 1(50%) |
| C.krusei                        | Sensitive | 1(100%) | 1(100%) | 1(100%) | 1(100%) | 1(100%) | 1(100%) | 1(100%) |
|                                 | Resistant | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| C.tropicalis                    | Sensitive | 1(100%) | 1(100%) | 1(100%) | 1(100%) | 1(100%) | 1(100%) | 1(100%) |
|                                 | Resistant | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| C.parapsilosis                  | Sensitive | 2(100%) | 2(100%) | 2(100%) | 2(100%) | 2(100%) | 2(100%) | 2(100%) |
|                                 | Resistant | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |

5.3. Laterality distribution

Otomycosis is commonly a one sided disease the above study is a proof to this fact. It is found in this study that 90% of cases had otomycosis in a single ear and only 10% of the patients had the disease in both ears. The above results are in accordance with studies by KO Paulose, Al Khalifa, P. Shenoy and RK Sharma who also reported that otomycosis is predominantly a unilateral disease.

5.4. Occupational incidence

In this study 45% of cases were Housewives comprising from surrounding villages and towns, they constituted major group because of cold damp working atmosphere of village kaccha houses. This group frequently clean and sweep the floor of the houses. The resulting dust containing fungal spores mix with air of the atmosphere as pre disposing agent for the initiation of the disease, Yehia et al (1990).

5.5. Seasonal incidence

In this study majority of cases were reported between April and November that constitute summer and rainy season in Hyderabad. Fungi abound in any soil or in sand which contains decomposing vegetable matter, droppings of cattle, goats and other domestic animals. This is dessicated rapidly in the tropical sun and dispersed as wind blown particles. The air borne fungal spores are carried on droplets of water vapour a fact which we believe correlates with the higher incidence during the above season. The above observations are in accordance with the study by Muglistan and G.O Donaghue in 1985 and Sood VP and Sinha A, Mohapatra LN (1964) and Beg MH, HH Bughari AT (1983).

5.6. Presenting complaints

All the patients in our study presented with itching (50%) and other common complaints were ear ache (22%), hearing loss (18%) and ear discharge (10%).

The above complaints and their incidence as mentioned earlier were in accordance with KO Paulose, Al Khalifa, P. Shenoy, RK Sharma et al (88%), Yehia MM and Alhabib HM and Shehab NM. (78%). Although pain tends to be the dominant complaint in bacterial infections, the most common complaint in otomycosis was severe itching sensation deep inside the canal, patients frequently report an irresistible urge to scratch the ear canal with the finger tip, or with any sharp instrument like hair pins.

5.7. Predisposing factors

In our study the predisposing factors taken into consideration were history of swimming or water entry into ear canal while bathing, normal or ritualistic dip in rivers and ponds, use of local antibiotic or steroid drops, trauma to external canal or any associated medical disease.
5.8. Role of cerumen
In our study all the cases had no cerumen in the external canal. This is accordance with study of KO Paulose and Al Khalifa, P.Shenoy RK Sharma3 (1989) and Youseff and Abdou MH,15 Ear wax contains numerous amino acids, saturated and unsaturated fatty acids which have an inhibitory effect on fungi (Senturia 1957).16

5.9. Otoscopic findings
In our study the most common otoscopic finding was the presence of black mycologic plug in 46% of patients, 17% patients presented with wet mycelial mat of fungal spores, 19% with dry mycelial mat, 13% as soft grayish white debris and 10% as cotton wooly mass.

These findings were in accordance with Youseff YA, and Abdou MH.15 (1962) 65% Sheikh et al 72% (1993) found that predominant mycological picture was of grayish white nature.

5.10. Associated ear disease
After adequate aural toilet various underlying changes in the canal wall and the nature of the tympanic membrane were noted. 41% of cases had congested tympanic membrane with edema of canal wall. 16% had CSOM and 12% had congested tympanic membrane, 4% had otomycosis with post mastoid cavity and no associated ear disease in 24%.

This is in accordance with K.O. Paulose et al3 (1989) who found 20% of otomycosis with CSOM and 4% in mastoid cavity infection. Silent perforation of tympanic membrane has been observed by Youseff YA and Abdou MH. (1962).15

5.11. Area Wise
In our study 86% of the cases were from rural area from surrounding villages. Where hot and humid climate containing dust with fungal spore mixed with air of the atmosphere act as the predisposing agent for the initiation of the diseases like poor hygiene, low socio economic status, malnutrition other factors include water in the ear, injury to the ear canal the above observation are in accordance with the study of preeti agarwal (2017).17

5.12. Fungal distribution
The fungal debris removed from the external auditory canal of all patients were subjected to 10% KOH slide preparation and examined for presence of fungal elements, those positive for fungal elements were also cultured on the sabourauds dextrose agar for fungal growth. In our study as pergillus Niger was isolated in 36% of cases, Aspergillus fumigatus in 9% of cases, Aspergillus flavus in 15%, Candida albicans in 11% and others in 8% of cases.

5.13. Treatment
5.13.1. Antifungal testing of aspergillus species by disk diffusion in different studies
In the present, antifungal susceptibility by disk diffusion showed that A. flavus, A. fumigatus, A.Niger were sensitive to all the 4 drugs listed.

Niger showed (5% resistance to itraconazole A flavus showed (6% to amphobhi

A study by Badee P el18 at has investigated and compared susceptibility pattern of 108 aspergillus species isolated from patients by CLSI ref broth microdilusion assay an E test it showed 90-100% sensitivity to all the drugs by all the species of aspergillus

5.13.2. Antifungal susceptibility
In the presence of antifungal susceptibility by candifast showed that C.Krusie, C parapsilosis, C tropicalis were sensitive to all of the 7 drugs listed.

Two isolates of C albicans (18.1%) were resistant to flu conazole and one isolate of C. Glabrata (50%) were resistant to flu conazole.

A study by Giri et al reported 100% agreement between disdiffusion and candifast when susceptibility pattern of C. Albicuns and NAC where compared and found candifast to be an easy and rapid method for identification and susceptibility testing of candida species.

6. Conclusion
It is concluded in this study that aspergillus species were most commonly isolated in patients presenting itching, ear ache and hearing loss, the most predominant being A.niger followed by A.flavus then A.Fumigatus also there were other like candida albicans, penicillum. Few cases showed normal ear flora. Antifungal susceptibility pattern of the study by disk diffusion revealed that A.Fumigatus, A.terreus were sensitive to the entire drug in the disk. A.flavus showed some resistance to the polyene antifungal. AFST pattern of the study by candifast kit revealed that C.Krussei, C.Tropicalis, C.parapsilosis were sensitive to all the seven drugs in the kit. C.glabrata and C.albicuns showed some resistance to the polyene and triazole group. Continued surveillance and laboratory confirmation by identification and speciation of causative agent routinely maybe valuable not only to treat the otomycosis symptoms effectively but also to use the antifungal appropriately so as to curb the emergence and spread of drug resistant aspergillus and candida species.

7. Acknowledgment
The author is thankful to Department of Microbiology and ENT for providing all the facilities to carry out this work. We acknowledge the extended support of our Dean and HOD of the college.
8. Conflicts of Interest
All contributing authors declare no conflicts of interest.

9. Source of Funding
None.

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Cite this article: Iqbal L, Jha KA, Kanaparthi AK. A study on changing trends of antifungal susceptibility in isolates from cases of otomycosis. IP Int J Med Microbiol Trop Dis 2021;7(1):41-48.