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A microbiological study of otomycosis

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Abstract

Aim of the study: To determine the fungi causing otomycosis and the associated bacterial pathogens in a rural set up in clinically suspected cases of otomycosis.

Methodology: Ear swabs were collected from 100 clinically suspected cases of otomycosis and from 50 persons who were apparently healthy. All the samples were processed by direct microscopy of KOH mount and Gram staining. Cultures were carried out for both fungal and bacterial isolates, which were identified by standard procedures. The data were statistically analysed.

Results: Among 150 samples, 51.3% yielded only fungal growth, 23.3% grew bacteria only and 19.3% showed mixed growth of fungi and bacteria. Major fungal isolates were Aspergillus spp. and Candida spp. One sample grew A. sydowii which is an uncommon agent causing otomycosis. Major bacterial isolates were Staphylococcus aureus and Pseudomonas spp. All pathogenic bacteria were sensitive to routinely used antibiotics. Samples taken from healthy persons did not yield any fungal or bacterial pathogens.

Keywords: Otomycosis, Aspergillus spp. Candida spp.

Introduction

Otomycosis or fungal otitis externa has typically been described as fungal infection of the external auditory canal with infrequent complications involving the middle ear.(1) It is more prevalent in warm and humid climates, particularly in monsoon (rainy) season,(2) the prevalence of otomycosis has been reported to be as low as 9% of cases of otitis externa and as high as 30.4% in patients presenting with symptoms of otitis or inflammatory conditions of the ear. Prevalence is also influenced by the geographical area, as otomycosis is most commonly present in tropical and subtropical humid warm climates.(3) The fungus may not be the primary cause but merely a secondary invader in cases of otitis externa. Therefore otomycosis can be seen in mixed fungal infection with bacterial infections.(4) The causative agents of otomycosis are various types of fungi, such as hyaline saprophytic molds, dematiaceous saprophytic molds, yeasts, and, rarely, pathogenic molds like dermatophytes.(5-9) Aspergillus and Candida spp. are the most frequently isolated fungi in patients with otomycosis.(10) Factors that predispose to otitis externa include absence of cerumen, high humidity, increased temperature, bacterial otitis externa, corticoid therapy, swimming(11) and local trauma –caused by sharp objects like sticks or hearing aids. Cerumen has a pH of 4 to 5 and so suppresses bacterial but conducive for fungal growth. Aquatic sports, including swimming and surfing, are particularly associated with otomycosis because repeated exposure to water results in removal of cerumen and drying of the external auditory canal.(12) Otomycosis is predominantly unilateral,(13) found in all age groups, but majority of the cases of otomycosis occur in patients aged 21 -30 years with equal male – female distribution.(14)

Sullia a small town of Dakshina Kannada District of Karnataka State. It is situated at the foothills of Western-Ghats, the climate is tropical with high humidity throughout the year, a condition conducive for fungal growth. Majority of the patients attending KVG Medical College Hospital at Sullia are agriculture labourers working in areca and rubber plantations.

No systematic study has been done to identify the agents causing otomycosis in this geographical area. Present study has been undertaken to determine the causative agents of otomycosis in patients attending ENT Department at this Medical College Hospital with ear ailments.

Material and Methods

A total of 150 patients with clinically suspected otomycosis, attending OPD of ENT department of K.V.G. Medical College Hospital were chosen for the study. Patients complaining of itching, pain, sensation of blockade in ear, impaired hearing or discharge from ear were selected. Another 50 age, sex matched apparently healthy persons without ear infection symptoms were used as controls. General information like age, sex, occupation, diabetic status, trauma, history of ear surgery or any fungal infection in other parts of body and laterality of symptoms were recorded. Any history of habits like use of oils/ear drops, wooden sticks or metal wax picks for removal of wax were also recorded. Otoscopic examination of external auditory meatus was carried out. From patients with suspected otomycosis three ear swabs were collected from affected ear. All samples were transported to the laboratory within half an hour for mycological and bacteriological examination. Ear swabs in triplicate were also collected from 50 healthy individuals and used as controls.

Processing

A portion of the scrapped material was suspended in 1-2 drops of 10% potassium hydroxide with methylene blue (2:1) on a clean slide and a cover glass was placed over it avoiding air bubbles. It was examined under microscope for the presence of fungal hyphae, conidiophores or yeast cells. Another portion of the scrapped material was used for Gram staining, and examined for presence of inflammatory cells, bacteria, yeast cells or other fungal elements.

The second swab was inoculated on two SDA slants with chloramphenicol. One tube was incubated at room temperature and another at 37ºC for 1 -2 weeks. Both tubes were observed for fungal growth daily. Fungal growth was identified by standard procedures.[15]

The third swab was inoculated on blood agar and MacConkey's agar plates, incubated at 37ºC for 24 – 48 hours and examined for bacterial growth. Identification of bacterial isolate was done by standard procedures.[16]

Ear swabs collected from control group were also processed by similar methods for fungal or bacterial growth.

Results

Age-wise distribution of study population– Distribution of cases of our study is provided in Table 1.

Table 1: Age-wise distribution of otomycosis cases

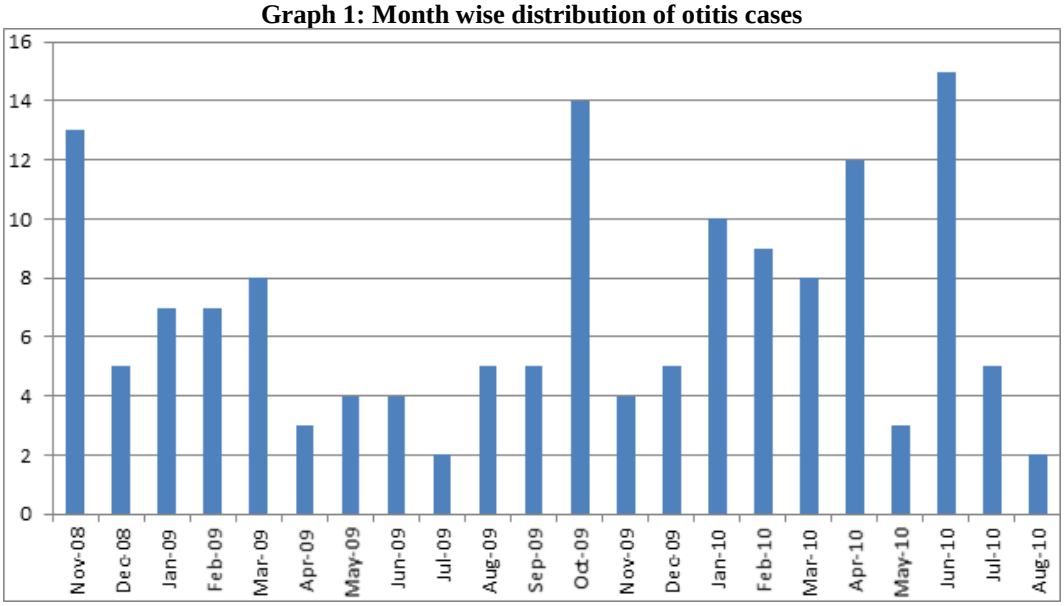
Age in years	Number of cases	Percentage
0 -10	21	14
11 - 20	32	21.3
21 -30	32	21.3
31 -40	22	14.7
41 -50	20	13.3
51 -60	09	6
>60	14	9.3

Out of 150 cases 64 cases were in 11 -30 years of age group constituting maximum of (42.6%) of the cases.

Sex distribution – There were 70 (46.7%) males and 80 (53.3%) females. There was a female predominance in otitis cases (53.3%).

Laterality – Out of 150 patients 70 (46.7%) had right ear involvement and 77 (51.3%) had left ear involvement. Only 3 (2%) had both ears involved. There is no significant difference in right or left ear involvement.

Graph 1: Month wise distribution of otitis cases



Peak numbers of cases were seen during June (15), October (14) and November (13). The peak was in June (rainy season), followed by October and November (post monsoon months).

Occupation – Out of the 150 cases of otomycosis 52(34.6%) were housewives, 42(28%) were farm workers and 36(24%) were students. Among others 13(8.6%) were officials and 7(4.6%) were children. Maximum number of cases were seen among housewives (34.6%) followed by farm workers (28%) and students (24%). Housewives are exposed to dusty air while cleaning the floors and also to the smoky atmosphere inside the kitchen. Since the weather here is humid even household work leads to excessive sweating. Further covering the head with clothing which blocks free movement of air adds to the already existing predisposition to otomycosis.

Contributing factors - Contributing factors for otomycosis included use of oils and ear drops in (32.6%) of cases. Use of wooden sticks, feather and metal wax picks also was seen in significant number of cases (36.5%). History of diabetes was seen in 4% of cases. Only one patient had history of apparent trauma. Twenty nine persons (19.33%), gave history of a combination of factors including using oils, ear drops and using sticks.

Signs and symptoms - Maximum number of patients (71.3%) complained of pain in the affected ear, followed by discharge from the ear (54.6%). Itching was predominant complaint among 49.3% of cases and 48% experienced a sense of blockade in the affected ear. Multiple complaints were given by most of the patients.

Direct Microscopy findings -Among 150 samples, 75(50%) showed only fungal elements, 33(22%) showed both bacteria and fungal elements and 34(22.6%) showed only bacteria. In total 108(72%) samples showed fungal elements and 67(44.6%) showed bacteria. Only 8 (5.3%) samples didn't show either fungal elements or bacteria on microscopy.

Culture - Results of fungal and bacterial cultures of 150 samples showed fungal growth alone in 77 (51.3%) samples, only bacterial growth in 35 (23.3%) cases and mixed bacterial and fungal growth in 29 (19.3%) cases. No growth could be detected in 9 cases at the end of 4 weeks of incubation (Table 2).

Among 108 samples showing fungal elements on KOH mount, 106 samples grew fungi in culture. Also among 67 samples showing bacteria on Gram stain, 64 samples grew bacteria in culture. There was very little difference in direct microscopy findings and culture results.

Table 2: Number of fungi and bacteria isolated from the study group

Number of cases	Type of growth	Fungi	Bacteria
58	Single fungus	58	-
19	Two fungi	38	-
16	One fungus, one bacteria	16	16
06	Two fungi, one bacteria	12	06
04	One fungus, two bacteria	04	08
02	Two fungi,two bacteria	04	04
01	Three fungi, one bacteria	03	01
33	Single bacteria	-	33
02	Two bacteria	-	04
141	Total	135	72
09	No growth	-	-

Among 150 samples, 58 samples yielded single fungus and 19 samples yielded two fungi each, without any bacterial isolates. Thirty-three samples grew single bacteria and two samples yielded two bacterial isolates each, without showing any fungal growth. All other samples yielded mixed fungal and bacterial growth. A total of 135 fungi were isolated from 86 samples and 72 bacterial isolates were grown from 42 samples. A total of 141 samples out of 150 samples yielded growth with 9 samples not showing any growth.

Fungal isolates - The various fungi isolated from study group is shown in Table 3

Table 3: Fungi isolated from cases of otomycosis

Fungi grown	Total number	Percentage
Aspergillus niger	67	49.63
Candida spp.	47	34.82
Aspergillus flavus	13	9.63
Penicillium spp.	03	2.22
Aspergillus fumigatus	02	1.48
Aspergillus sydowii	01	0.74
Cladosporium spp.	01	0.74
Fusarium spp.	01	0.74
Total	135	100

The commonest isolate was Aspergillus niger constituting 49.63% of fungal isolates. Candida spp. constituting 34.82% was the second commonest isolate. With 13 isolates of A. flavus, 2 isolates of A. fumigatus and 1 isolate of A. sydowii, Aspergillus spp. made up for 61.48% of fungal isolates. Penicillium spp. constituted 2.22%, Cladosporium and Fusarium spp. constituted 0.74% each. Aspergillus spp. is the commonest fungus found in the atmosphere and a known contaminant everywhere. Conidia of these fungi spread through the air everywhere and wherever it finds suitable site for its growth it grows. When there is a predisposing condition for fungal growth in the external auditory canal, it is the commonest fungus to colonize and grow there.

Bacterial isolates - The various bacteria isolated from the study group is depicted in Table 4

Table 4: Bacterial isolates grown among study group

Bacteria	Total number	Percentage
Staphylococcus aureus	21	29·17
Pseudomonas spp.	20	27·78
CONS	14	19·45
Klebsiella spp.	07	9·72
Proteus spp.	03	4·17
Escherichia coli	03	4·17
Enterobacter spp.	02	2·78
Citrobacter spp.	01	1·38
Morganella spp.	01	1·38
Total	72	100

The major bacterial isolate was *Staphylococcus aureus* (29.17%), followed by *Pseudomonas spp.* (27.78%) and CONS (19.45%). *Klebsiella spp*. (9.72%), *Proteus spp*. (4.17%), *Escherichia coli* (4.17%), *Enterobacter spp*. (2.78%), *Citrobacter spp*. (1.38%)

and *Morganella spp.* (1.38%) are the other isolates. A total of 72 bacteria were isolated from 42 samples.

Antibiotic susceptibility of bacterial isolates: The antibiotic susceptibility pattern of isolated bacterial isolates is depicted in Table 5.

Table 5: Antibiotic susceptibility pattern of bacterial isolates from the study group

Bacteria	Antibiotics										
	G	**Ak**	**Cx**	**A**	**Cp**	**Az**	**Ac**	**Sp**	**Co**	**Cd**	**Ce**
Staphylococcus aureus(21)	10		09	07	14	15					
Pseudomonas spp.(20)		10					04	14	05	16	05
CONS (14)	08		07	06	09	09					
Klebsiella spp.(7)	06				02		02	04	04		04
Escherichia coli (3)		03			02		02	02	02		02
Proteus spp. (3)		02			03		02	01	02		02
Enterobacter spp. (2)		01			02		01	01	02		01
Morganella spp. (1)		01			01		01		01		01
Citrobacter spp. (1)		01						01	01		

G=Gentamycin Ak=Amikacin Cx=Ciprofloxacin A=Ampicillin Cp=Cephalaxin Az=Azithromicin Ac=Amoxyclave Sp=Sparfloxacin Co=Cotrimoxazole Cd=Clindamicin Ce=Cephotaxime.

The commonest bacterial isolate, S.aureus was sensitive to azithromicin, ciprofloxacin and gentamycin among most isolates. Pseudomonas spp. was mostly sensitive to ceftazidime and amikacin. Klebsiella spp. was sensitive to gentamicin in most instances and other isolates were sensitive to amikacin among most isolates (Table 5).

Control Group

Among 50 samples from healthy individuals, 26 samples were from individuals aged between 21 – 30 years. Ages of other people varied between 0 – 60 years. Among them 38 (76%) were females and 12 (24%) were males. All these samples yielded growth of nonpathogenic bacteria like diphtheroids. No fungus could be isolated from these samples.

Statistical Analysis

On application of chi-square test –

There was significant difference between KOH mount microscopy findings in cases (72%) and controls (Nil) – P=0.000, highly significant.

There is significant difference between fungal growth in cases (70.6%) and controls (6%) –P=0.000, highly significant.

There is significant difference between pathogenic bacteria in cases (42.6%) and controls (Nil) – P=0.000, highly significant.

There was statistically significant correlation between –

1. Ear discharge and bacterial growth, 86%, P<0.05.
2. Ear blockade and fungal growth, 81%, P<0.05.
3. Itching in ear and KOH microscopy positivity, 85%, P<0.05.
4. Use of oil into ears and fungal growth in culture, 89%, P<0.05.

Discussion

Otomycosis is a chronic superficial fungal infection that affects the deeper ear canal skin and the tympanic membrane. Unless manifested in the classical way, they tend to be misdiagnosed. Otomycosis is commonly associated with increased ear canal moisture, warmth and treatment of a bacterial infection with long term topical antibiotic therapy, which can lead to depletion of the protective cerumen layer, maceration of underlying skin, increase in ambient pH and a modification of the microbial flora of external auditory canal, thereby selecting for untreated organisms. A medical history of diabetes and / or an immunocompromised state may be present.[17] Otomycosis is predominantly unilateral both in males and females.[18]

Sullia is a township situated at the foothills of Western Ghats with high humidity throughout the year. There is heavy rainfall from June to September, sometimes stretching beyond October. People are commonly engaged in agricultural work, in areca and rubber plantations.

Present research was undertaken at K.V.G. Medical College Hospital, Sullia, in order to identify the causative agents of otitis externa in this area. Ear swabs were collected from 150 patients attending ENT OPD with clinically suspected otitis and processed in Microbiology diagnostic laboratory for fungi and bacteria.

Among 150 samples, we could grow fungi from 77 (51.3%) samples, bacteria from 35 (23.3%) samples and a mixture of bacteria and fungi from 29 (19.3%) samples. No growth could be observed in 9 (6%) samples. In total 112 (74.6%) samples grew pathogens in culture. This is in comparison to the study conducted by Kaur et al.[19] Other Indian studies report varying culture positivity from 28% to 100%.[20-23] Higher incidence of otomycosis in this area may be due to hot and humid weather prevailing throughout the year and heavy rainfall during monsoon.[18]

In our study majority of patients belonged to 11 -30 years of age. This is comparable to those observed by Kumar et al in their study.[24] Other Indian studies report high incidence of otomycosis among 21 – 30 year age group.[13,18,25-28] This is because, people of younger age group spend more time outside and are exposed to open air containing spores of locally prevalent fungi.[19]

More female patients had otomycosis in our study in comparison to males. This is in comparison to some Indian studies.[21,27,29,30] More male patients with otomycosis is also reported in many Indian studies.[13,18,20,26] These differences are attributed to various factors like, covering head with cloth, working in dusty and smoky atmosphere.[30]

In our study maximum numbers of cases were seen during the period of heavy rainfall, namely June, October and November. Otomycosis has a greater prevalence in hot, humid and dusty atmosphere of tropics and subtropics.[13]

Occupation of the patients also contributes to the incidence of otomycosis. Majority of patients in our study were housewives (34.6%). Field workers constituted 28% of patients. These people are exposed to dust while cleaning the floors and also work in windy fields where they are constantly exposed to dust containing fungal spores. Outdoor labourers of low socioeconomic status working in high humid, hot and dusty environment are more prone for otomycosis.[19] Otomycosis is predominantly unilateral.[13] In our study 98% of patients had unilateral otomycosis. Only 2% of cases showed bilateral involvement. There is no significant difference in right or left ear involvement. This is in comparison to other Indian studies.[19,29]

There are various contributing factors for otomycosis. Majority of our patients gave history of using oil and ear drops. Second contributing factor was introducing feathers and sticks into the external ear. Diabetes contributed in 4% of cases. One patient gave history of trauma. The etiological factors predisposing to otomycosis are, long term use of antibiotic or steroid ear drops, use of oil, maceration of underlying skin and increase in ambient pH.[31] Swimming, yeast or fungal infections in other parts of the body, diabetes mellitus and immunocompromised state are other predisposing factors.[26]

Most of the patients complained of pain in the affected ear in our study. Most Indian studies report, itching as the major symptom among otomycosis patients.[13,18,23,26,27] Earache was the major symptom in some other studies.[22,25,31] In our study, most patients presented with more than one symptom.

Among 150 samples 72% showed fungal elements on microscopy and 74.6% of samples were culture positive. This is comparable to the study by Kondity et al[32] at Karnool where 77.06% of samples showed fungal elements in microscopy and 79.82% of samples were culture positive. Gokhale et al[25] from Bagalkot, reported fungal elements in 82.52% samples on microscopy and 85.44% of samples by culture. Other Indian studies report positive cultures varying from 28% to 100%.[20-25] While 44.67% of samples were positive for bacteria by microscopy of the Gram stained smear, only 42.67% samples grew bacteria. There was a wide variation in the outcome of bacterial cultures of ear discharges among Indian studies. In our study, 51.3% samples yielded only fungi, 23.3% samples yielded only bacterial growth and 19.33% samples gave mixed bacterial and fungal growth. Kumar et al[24] could isolate bacteria only from 69% of samples and mixed bacterial and fungal growth from 6% of samples. Panchal et al[21] isolated only fungi from 46% of cases and mixed bacterial and fungal growth from 26.08% of cases. Kailash et al[33] isolated bacteria from 61.73% of cases and mixed growth in 38.27% of cases.

The commonest fungal isolate in our study was Aspergillus spp. (61.48%) followed by Candida spp. (34.82%). Among Aspergillus spp. the commonest species isolated was *A. niger* (49.63%). Majority of Indian studies on otomycosis report Aspergillus spp. as the commonest isolate with percentages varying from 40 to 89.3%.[19-26] Among these studies the major Aspergillus species isolated was *A. niger*.[20-23,25,26] Candida Spp. was isolated as the second commonest isolate in most of Indian studies.[19,21-26] Further, the isolation of different fungal species varies from place to place depending upon the geographical pattern of fungus in that area.[34] Aspergillus spp. and Candida spp. are the commonest isolates in otomycosis.[35,36] The commonest species isolated among them are *Aspergillus niger* and *Candida parapsilosis*.[37] *Aspergillus niger* grows on cerumen, epithelial scales and detritus deep in external canal (Fig. 1). The lesion of aspergillosis is dry. Occurrence of exudate and foul smell is indicative of a bacterial etiology.[38] *Candida albicans* has been said to be a causative agent of otomycosis.[39] It can colonize mouth, vagina and gut, but not on skin.[40] But other species of candida are normal flora of skin and are frequently blamed as causative agents of otomycosis, but their role is debatable as mentioned in some studies.[41-43] There are studies reporting histopathological evidence of infection and also Candida spp. as the sole pathogen causing otomycosis.[19,37,44] Further, Candida spp. is said to be mainly responsible for otomycosis in immunocompromised hosts.[45]

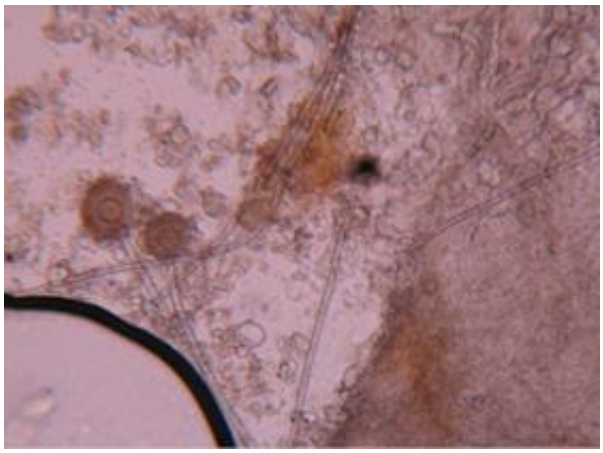


Fig. 1: A.niger conidia in cerumen

Aspergillus sydowii (Fig. 2) is a saprophytic fungus found in soil that can contaminates food and is occasionally pathogenic to humans.[46] It is found in Wheat Qu, a source of microorganisms for Chinese rice wine brewing.[47] It is a pathogen of sea fan corals of Caribbean, in warm temperatures.[48] A massive central Australian dust storm in September 2009 was associated with abundant fungal spores and hyphae of *A. sydowii*, in coastal waters between Brisbane and Sydney.[49] *A. sydowii* has been isolated from cases of onychomycosis,[50] Rhinosinusitis[51] and from root canals with pulp necrosis[52] in humans. It was also isolated from a case of peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis.[53] Further, *A. sydowii* has been found to be one of the causative agents of invasive fungal infections among transplant patients.[54] *A. sydowii* has been reported to cause dermatitis in a thoroughbred horse.[55] We are reporting here otomycosis caused by *A. sydowii* in one patient. It was clinically diagnosed as eczema of external auditory canal. Following treatment failure, ear swabs were collected and fungal culture yielded Aspergillus spp. which was identified as *Aspergillus sydowii* on morphological features. In a study by Hubka et al, among 178 isolates of various Aspergillus species, from Czech patients, 17 (9.6%) belonged to the species *A.sydowii*. Among them 3 isolates were isolated from cases of external otitis, constituting 9.4% of Aspergillus spp. isolates causing otitis externa.[56] No Indian studies report isolation of *A. sydowii* from cases of otomycosis. This is the first report of *A. sydowii* causing otomycosis in India. Other fungi isolated in this study, like Penicillium spp. and Cladosporium spp. are also isolated in Indian studies.[19,23,27] Fusarium spp. is one among the rare isolates from external auditory canal.[10]

Fig. 2: A.Sydowii under lactophenol cotton blue mount

Among bacteria, Staphylococcus aureus and Pseudomonas spp. were the predominant isolates. These organisms were isolated from normal external auditory canal also. Trauma, exposure to water, persistent ear discharge and seborrhea were considered to be the predisposing factors for infection with these bacteria.[57] S. aureus and Pseudomonas spp. are the major isolates from cases of external otitis in Indian studies.[24,33,58] Mixed bacterial and fungal infections were also reported in Indian studies.[21,24,33,58] Bacterial infection of auditory canal may be one of the predisposing factors for development of otomycosis. Further, use of antibiotic steroid drops for treating external otitis may predispose for fungal growth.[4] No pathogenic bacteria or fungus were isolated from control group. All major pathogenic bacteria were sensitive to routinely used antibiotics like, aminoglycosides and fluoroquinolones.

Conclusion

Prevalence of otomycosis is higher in hot and humid weather and more cases are seen during rainy season. The incidence is more in 11- 30 years of age with a female predominance, mostly among housewives. Mostly otomycosis is unilateral. Manipulation of external ear with hard objects is the major reason for growth of fungi in auditory canal. Otalgia was the presenting symptom in majority of patients. Aspergillus spp. and Candida spp. are the major fungal isolates and S.aureus and Pseudomonas spp. are major bacterial isolates. Upon clinical suspicion culture of both bacteria and fungi are required to identify the pathogen, as mixed infections are also seen in cases of external otitis. Fungal infection of the external canal should be suspected in all chronic cases of otitis externa which do not respond to conventional topical therapy.

Prevention: Avoidance of use of oil or similar substances into the ear and also avoiding introduction of any hard objects like ear picks or sticks into the ear are important in preventing external otitis. These hard

objects traumatize the skin of the external auditory canal predisposing it to bacterial and fungal infections. However cleaning the external auditory canal free from debris is also important, which may be achieved by using soft cloth or clean cotton buds. Keeping the ear dryand clean without traumatizing the skin and avoiding entry of moisture into the ear canal go a long way in preventing external otitis, either fungal or bacterial.

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