Assessment of Risk Factors and Microbial Profile of Otomycosis in Patients Attending Tertiary Level Hospital of Western Rajasthan, India

VISHAKHA ASHOPA¹, USHA VERMA², POOJA NAREDA³, ESHANK GUPTA⁴, PRABHU PRAKASH⁵

(CC) BY-NC-ND

ABSTRACT

Microbiology Section

Introduction: Otomycosis or fungal otitis externa is a superficial, subacute or chronic infection of External Auditory Canal (EAC) with irregular complications involving the middle ear, which is characterised by inflammation, pruritus, scaling, otalgia, fullness of ear, tympanic membrane perforation, hearing impairment and ear discharge. Fungi can either be the primary pathogen or be superimposed on bacterial infections.

Aim: To identify causative agents of otomycosis and to determine the associated predisposing factors.

Materials and Methods: The present study was a hospital based prospective study which was conducted from April to September 2019 in Department of Microbiology and Department of Ear Nose and Throat (ENT) in a tertiary level hospital. Total 150 samples was collected from clinically diagnosed patients of otomycosis of any age group. Identification of fungal and bacterial isolates were done by standard microbiological procedures, and demographic profile, occupation, predisposing factors were evaluated. Categorical data was presented as frequencies and percentages. The association between the

pre-disposing factors (Ear pricking, oil instillation) and the prevalence of otomycosis was analysed by chi-square test. The p-values <0.05 was considered significant.

Results: Male to female ratio in present study was 1:1.30, females were more than males. Maximum cases were in the age group of 21-30 years i.e., 37 (24.67%), occupation wise maximum cases were seen in housewives 55 (36.67%). Ear pricking was the most common predisposing factor leading to otomycosis i.e., 122 (81.33%). *Aspergillus* species and *Candida* species were the most commonly identified fungal pathogens in otomycosis 76 (70.37%) and 17 (15.74%), respectively. In bacterial isolates *Pseudomonas* species 20 (13.33%) was the most common causative agent, cases of mixed bacterial and fungal infection were also seen.

Conclusion: In the present study, *Aspergillus* species was found to be the most common fungi involved in otomycosis. There is need to educate the community about the common practice of ear pricking and instilling oil in ear which leads to otomycosis.

Keywords: External auditory canal, Fungal otitis externa, Lactophenol cotton blue stain, Sabouraud's dextrose agar

INTRODUCTION

Otomycosis or fungal otitis externa is a superficial, subacute or chronic infection of External Auditory Canal (EAC) with irregular complications involving the middle ear [1], which is characterised by inflammation, pruritus, scaling, otalgia, fullness of ear, tympanic membrane perforation, hearing impairment and ear discharge. In study done by Fasunla J et al., and Pontes ZB et al., concluded that otomycosis prevalence depends upon different climatic conditions with higher number of cases in the hot, humid and dusty areas of the tropics and subtropics [2,3]. In India otomycosis is a common medical problem and health hazard [4,5]. There are many predisposing factors of otomycosis which may be systemic or local. Long term use of antimicrobial agents, immuno-compromised status or comorbid conditions like diabetes are systemic predisposing factors of otomycosis while high humidity in EAC, accumulation of epithelial debris or instrumentation or trauma caused by the use of unsterile sharp or pointed objects like key, hair pins and match sticks etc. may be local predisposing cause of otomycosis [6].

Fungi can either be the primary pathogen or be superimposed on bacterial infections [7]. There are wide spectrum of fungi which leads to otomycosis but *Aspergillus niger* and *Candida albicans* are most common offenders [4,5,8].

The present study was conducted with the aim to identify both fungal and bacterial agents which causes otomycosis and to determine the associated risk factors.

Journal of Clinical and Diagnostic Research. 2020 Feb, Vol-14(2): DC01-DC04

MATERIALS AND METHODS

The present study was a hospital based prospective study which was conducted from April to September 2019 in the Department of Microbiology at Umaid Hospital, Dr. SNMC Jodhpur, Rajasthan, India. Total 150 samples were collected.

Inclusion criteria: Clinically diagnosed patients of otomycosis with symptoms like itching, pain, feeling of blocked ear, tinnitus, deafness, discharge and otoscopic findings revealing wet or dry matted masses of hyphae or white cheesy material and those who gave their written consent, attending ENT outpatient department at MDM hospital, Dr SNMC, Jodhpur.

Exclusion criteria: Patient who refused to give their consent.

Institutional Ethical Clearance was taken and ethical certificate reference number is SNMC/IEC/2019/PLAN/204.

To evaluate and analyse demographic profile (age, sex, and occupation), predisposing factors, presenting complaints, a predesigned proforma was used in clinically diagnosed patients of otomycosis.

Two ear swabs were collected aseptically. One ear swab was used for direct microscopy (Gram's staining, 10% KOH and wet mount) and second swab was used for culture (mycological and bacteriological culture). For fungus culture, Sabouraud's Dextrose Agar (SDA) with chloramphenicol (Himedia, India), was used as selective medium [9]. Ear swabs were inoculated on two slants

of SDA with chloramphenicol and incubated at 25°C in Biological Oxygen Demand (BOD) incubator and at 37°C incubator for four weeks and slants were examined at regular interval for fungal growth. In case of any filamentous fungal growth, Lactophenol Cotton Blue (LPCB) mount and microscopy was done for identification of filamentous fungal isolate. For characterisation of Candida isolates, colonies were inoculated on HiChrome agar (Himedia, India) for species identification [10].

For aerobic pyogenic identification, sample was cultured on Blood Agar and MacConkey Agar for 48 hours and examined for bacterial growth. Identification of the bacterial isolates was done by standard bacteriological procedures [11].

STATISTICAL ANALYSIS

The prevalence of otomycosis was analysed with the chi-square test using SPSS version 25 and p values <0.05 were considered significant. Categorical data were presented as frequencies and percentages.

RESULTS

Based on clinically diagnosed patients of otomycosis according to complaints. Sensation of blocked ear 99 (66%) and itching 50 (33.33%), were maximum while minimum complaint were noted in ear discharge and in tinnitus which were 13 (8.66%) and 21 (14%) respectively [Table/Fig-1]. In male to female ratio there was pre dominance in female patients as compared to males in a ratio of 1:1.30 (65:85). While in urban population females were affected more in comparison to males in a ratio of 1.34:1 [Table/Fig-2]. The maximum cases were found in the age group of 21-30 years i.e., 37 (24.67%) while minimum cases were detected in the age group of >60 years i.e., 10 (6.66%) [Table/Fig-3]. Out of 150 cases 117 (78%) patients were from urban background in compare to rural population in which 33 (22%) patients were engaged for this study. Maximum cases were housewives 55 (36.67%) followed by student 51 (34.00%), farmer 12 cases (8%) while minimum cases were seen in auto driver, cobbler, engineer, mechanic, shopkeeper, supervisor, welding and handicraft workers (1 case in each) [Table/Fig-4].

	Clinical manifestation	Yes	No		
1	Itching	50 (33.33%)	100 (66.66%)		
2	Blocked ear	99 (66%)	51 (34%)		
3	Ear pain	84 (56%)	66 (44%)		
4	Ear discharge	13 (8.66%)	137 (91.33)		
5	Tinnitus	21 (14%)	129 (86%)		
[Table/Fig-1]: Distribution according to patients complaints.					

Area Sex	Urban	%	Rural	%	Total
Male	50	33.33%	15	10.00%	65
Female	67	44.67%	18	12.00%	85
Total	117	78.00%	33	22.00%	150
[Table/Fig-2]: Demographic distribution of cases.					

SI. No.	Age (years)	Male	Female	Total	%
1	<10	12	10	22	14.67
2	11-20	14	13	27	18.00
3	21-30	16	21	37	24.67
4	31-40	09	20	29	19.33
5	41-50	03	10	13	08.67
6	51-60	05	07	12	08.00
7	>60	06	04	10	06.66
Total		65	85	150	100
[Table/Fig-3]. Distribution of causative agent in relation to age and sex					

SI. No.	Occupation	No	%	
1	Student	51	34.00	
2	Auto driver	01	00.67	
3	Businessman	12	08.00	
4	Carpenter	02	01.33	
5	Cobbler	01	0.67	
6	Engineer	01	0.67	
7	Factory worker	02	01.33	
8	Farmer	12	08.00	
9	Handicraft worker	01	00.67	
10	House wife	55	36.67	
11	Labour	03	02.00	
12	Mechanic	01	00.67	
13	Shopkeeper	01	00.67	
14	Supervisor	01	00.67	
15	Teacher	01	00.67	
16	Welding worker	01	00.67	
[Table/Fig-4]: Occupation wise distribution.				

Ear pricking, use of local and systemic antimicrobial agents and oiling were most common predisposing factors leading to otomycosis i.e., 122 (81.33%), 37 (24.67%) and 33 (22%), respectively. While minimum cases were seen in diabetic patients i.e., 02 (1.33%) and in this study all patients were HIV negative [Table/Fig-5].

SI. No.	Predisposing factors	No. of cases	%		
1	Ear pricking	122	81.33		
2	Oiling	33	22.00		
3	Swimming	13	08.67		
4	Use of local and systemic antimicrobials	37	24.67		
5	Diabetes	02	01.33		
6	Any history of discharge	13	08.67		
[Table/F	[Table/Fig-5]: Predisposing factors.				

In present study, maximum number of mould enrolled were Aspergillus niger i.e., 54 (50%) followed by Aspergillus fumigatus 10 (9.25%), Aspergillus flavus 08 (7.40%), Aspergillus terreus 04 (3.70%). While minimum cases were enrolled in Scopularasis spp. 1 (0.92%) followed by Rhizopus microsporus, Rhizomucor spp., Syncephalastrum spp., Basidiobolus spp., Paecilomyces spp. (1 in each) [Table/Fig-6].

In dematiaceous fungi, Chrysosporium spp. was associated with 01 (0.92%), Alternaria spp. 01 (0.92%), Bipolaris spp.01 (0.92%), Cladosporium spp. 01 (0.92%), Curvularia spp.01 (0.92%).

In yeast like fungi, Candida krusei were isolated from 14 (12.96%), Candida albicans 01 (0.92%), Candida dubliniensis 01 (0.92%), Candida parapsilosis 01 (0.92%).

In aerobic pyogenic bacteria, Pseudomonas spp. was most common isolate in 20 (13.33%) cases, Klebsiella spp. in 11 (10.1%) cases, Escherichia coli in 01 (0.67%) and Staphylococcus aureus were present in 02 (2.38%) cases.

DISCUSSION

Agarwal P and Devi LS and many other researchers concluded high prevalence of fungal otitis externa in tropical and subtropical regions of the world including India in the last few decades [1-4,6,8,9,12].

Occurrence of otomycosis was high in present study 108 (72%) which is similar to the studies conducted by Kaur R et al., and Barati B et al., who reported 74.6% and 69%, respectively [5,13]. As fungi are found abundantly on decayed plant matter and are saprophytic in nature, workers in moldy or dusty environment are usually affected

Vishakha Ashopa et al., Assessment of Risk Factors and Microbial Profile of Otomycosis in Patients Attending Tertiary Level Hospital

SI. No.	Fungal isolate	Number (%) of samples positive for fungi, n=108	Associated bacterial isolates (n)		
	Aspergillus niger	54 (50%)	Pseudomonas spp.	6	7.14%
			Klebsiella spp.	4	4.76%
			CONS	12	14.28%
			<i>Micrococcus</i> spp.	01	1.19%
1	Aspergillus flavus	00 (7.400/)	Klebsiella spp.	02	2.38%
		08 (7.40%)	CONS	01	1.19%
	Aspergillus fumigatus	10 (9.25%)	<i>Micrococcus</i> spp.	01	1.19%
			CONS	02	2.38%
	Aspergillus terreus	4 (3.70%)	Klebsiella spp.	01	1.19%
2	Rizomucor spp. Rhizopus Microsporus	1 (0.92%) 1 (0.92%)	-	-	-
3	C. albicans C.dubiliensis C. krusei C. parapsilosis	1 (0.92%) 1 (0.92%) 14 (12.96%) 1 (0.92%)	Pseudomonas spp.	02	2.38%
			CONS	03	3.57%
4	Alternaria spp. Bipolaris spp. Cladosporium spp. Curvularia spp. Crysosporium spp. Syncephalastrum spp. Basidiobolus spp. Paecilomyces spp. Scopularasis spp.	1 (0.92%) 1 (0.92%) 1 (0.92%) 1 (0.92%) 1 (0.92%) 3 (2.77%) 1 (0.92%) 1 (0.92%) 1 (0.92%) 1 (0.92%)	Pseudomonas spp.	01	1.19%
			CONS	03	3.57%
	Mixed Fungal Infection	With 2 fungi Aspergillus spp.: 10{(9.25%) (<i>A.niger</i> complex, <i>A.fumigatus</i> complex, <i>A.flavus</i> complex} <i>Candida</i> spp3 (2.77%)	CONS	01	1.19%
5			Pseudomonas spp.	01	1.19%
		With no fungi (48)	Staph.aureus	02	2.38%
6			CONS	24	28.57%
			Micrococcus spp.	02	2.38%
			E. coli	01	1.19%
			Pseudomonas spp.	10	11.90%
			Klebsiella spp.	04	4.76%

[Table/Fig-6]: Spectrum of isolates among patient

more. The higher cases of otomycosis may be due to humidity [9]. In this study, ratio of females 85 (56.66%) were more as compared to males 65 (43.33%) which is in concordance with the studies done by Fasunla J et al., Pontes ZB et al., Barati B et al., and Yehia MM et al., who reported predominance in female cases 233 (61.64%), 12 (60%), 86 (50.3%), 65.4%, respectively [2,3,13,14].

In the present study, the incidence of infection was seen in all age groups but in age group 21-30 years incidence was 24.67%, followed by 31-40 years 19.33% [Table/Fig-3] which is similar to the studies conducted by Prasad SC et al., and Agarwal P and Devi LS who reported 21.33% and 66.31%, respectively [1,9]. Out of 150 samples, maximum cases were seen in housewives 55 (36.67%) which is similar to the study conducted by Adoga AS and Iduh AA who reported 28.60% [15], while by the studies conducted by Prasad SC et al., maximum cases were seen in patients who were indulged in agriculture 70% and Agarwal P and Devi LS reported maximum cases in outdoor farmers and labours who were exposed to fungal spores together with dust owing to their working conditions [1,9].

In all the patients of otomycosis, predominant symptoms were itching, blocked sensation of the ear; there was complaint of ear

discharge, ear pain and tinnitus also. The incidence of otomycosis was high in patients with history of ear pricking (81.33%) habit of cleaning ear with contaminated objects such as key, hair pin etc., leads to inoculation of fungal debris in EAC which is similar to the study conducted by Pontes ZB et al., [3] while, instilling coconut oil into the ear was the most common i.e., 42% causative agent in the study done by Prasad SC et al., [1]. The use of topical antibiotic ear drops and use of systemic antimicrobial agents was in 24.67% and the habit of instilling oil in the ear was recorded in 22% cases.

In study done by Prasad SC et al., *Aspergillus* spp. were isolated in 80% cases, *Penicillium* spp. in 8%, *C.albicans* 4%, *Rhizopus* spp. and *Chrysosporium* spp. in 1% each [1]. In fungal bacterial association in the study group, aerobic pyogenic bacteria were isolated as *Pseudomonas* spp. in 20 (13.33%) cases similar to the study done by Singer DE et al., [16].

In this study, *Aspergillus* spp. and *Candida* spp. were the most commonly identified fungal pathogens in otomycosis which is similar to the studies conducted by Agarwal P and Devi LS, Aneja KR et al., and Hagiwara S et al., [9,17,18].

Although antifungal agents are effective in eradicating infection and removing the predisposing factors have to be taken into consideration. Most of the fungi thrive in moist atmosphere and in the presence of epithelial debris; tropical drugs do not act unless these debris and discharge is removed. Hence careful drying and cleaning of EAC and personal hygiene is recommended in treatment of otomycosis.

Limitation(s)

Anaerobic culture, molecular and genetic identification of isolates were not done due to non-availability of facilities.

CONCLUSION(S)

Fungal otitis externa is seen highly prevalent in tropical and subtropical regions of the world. In the present study, *Aspergillus* spp. were found to be the most common fungi involved in otomycosis along with other rare fungi like *Candida* spp., *Rhizomucor*, Dematiaceous fungi with bacterial co-infection by *Pseudomonas* spp. and other bacterial isolates. As ear pricking was most common pre-disposing factor leading to otomycosis, there is need to educate community about its serious consequences.

REFERENCES

- Prasad SC, Kotigadde S, Shekhar M, Thada ND, Prabhu P, D'Souza T, et al. Primary otomycosis in the Indian subcontinent: Predisposing factors, microbiology, and classification. Int J Microbiol. 2014;2014:636493.
- [2] Fasunla J, Ibekwe T, Onakoya P. Otomycosis in western Nigeria. Mycoses. 2008;51(1):67-70.
- [3] Pontes ZB, Silva AD, Lima EdeO, Guerra Mde H, Oliveira NM, Carvalho Mde F, et al. Otomycosis: Retrospective study. Braz J Otorhinolaryngol. 2009;75(3):367-70.
- [4] Viswanatha B, Sumatha D, Vijayashree MS. Otomycosis in immunocompetent and immunocompromised patients: Comparative study and literature review. Ear Nose Throat J. 2012;91(3):114-21.
- [5] Kaur R, Mittal N, Kakkar M, Aggarwal AK, Mathur MD. Otomycosis: A clinicomycologic study. Ear Nose Throat J. 2000;79(8):606-09.
- [6] Ho T, Vrabec JT, Yoo D, Coker NJ. Otomycosis: Clinical features and treatment implications. Otolaryngol Head Neck Surg. 2006;135(5):787-91.
- [7] Hurst WB. Outcome of 22 cases of perforated tympanic membrane caused by otomycosis. J Laryngol Otol. 2001;115(11):879-80.
- [8] Pradhan B, Tuladhar NR, Amatya RM. Prevalence of otomycosis in outpatient department of otolaryngology in Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Ann Otol Rhinol Laryngol. 2003;112(4):384-87.
- [9] Agarwal P, . Otomycosis in a rural community attending a tertiary care hospital: Assessment of risk factors and identification of fungal and bacterial agents. J Clin Diagn Res. 2017;11(6):DC14-18.
- [10] Chander J. Textbook of Medical Mycology. 3rd Edition. New Delhi: Mehta Publishers; 2002.
- [11] Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Medical Microbiology, Collee JG, Fraser AG, Marmion BP, Simmons A., editors. Mackie and McCartney's Practical. New York NY, USA: Churchill Livingstone; 1996. pp. 131-145.

Vishakha Ashopa et al., Assessment of Risk Factors and Microbial Profile of Otomycosis in Patients Attending Tertiary Level Hospital

- [12] Mgbor N, Gugnani H. Otomycosis in Nigeria: Treatment with mercurochrome. Mycoses. 2001;44(9-10):395-97.
- [13] Barati B, Okhovvat SAR, Goljanian A, Omrani MR. Otomycosis in Central Iran: A clinical and mycological study. Iran Red Crescent Medical J. 2011;13(12):873-76.
- [14] Yehia MM, Al-Habib HM, Shehab NM. Otomycosis: A common problem in North Iraq. J Laryngol Otol. 1990;104(5):387-89.
- [15] Adoga AS, Iduh AA. Otomycosis in Jos: Predisposing factors and Management. Afr J Med Med Sci. 2014;43(Suppl 1):209-13.
- [16] Singer DE, Freeman E, Hoffert WR, Keys RJ, Mitchell RB, Hardy AV. Otitis externa: Bacteriological and mycological studies. Ann Otol Rhinol Laryngol. 1952;61(2):317-30.
- [17] Aneja KR, Sharma C, Joshi R. Fungal infection of the ear: A common problem in the north eastern part of Haryana. Int J Ped Otorhinolaryngol. 2010;74(6):604-07.
- [18] Hagiwara S, Tamura T, Satoh K, Kamewada H, Nakano M, Shinden S, et al. The molecular identification and antifungal susceptibilities of *Aspergillus* species causing otomycosis in Tochigi, Japan. Mycopathologia. 2019;184(1):13-21.

PARTICULARS OF CONTRIBUTORS:

- 1. Senior Demonstrator, Department of Microbiology, Dr SNMC, Jodhpur, Rajasthan, India.
- 2. Senior Demonstrator, Department of Microbiology, Dr SNMC, Jodhpur, Rajasthan, India.
- 3. Resident, Department of Microbiology, Dr SNMC, Jodhpur, Rajasthan, India.
- Senior Demonstrator, Department of Microbiology, Dr SNMC, Jodhpur, Rajasthan, India.
 Professor, Department of Microbiology, Dr SNMC, Jodhpur, Rajasthan, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Prabhu Prakash,

3KA 9, First Puliya, CHB, Jodhpur, Rajasthan, India. E-mail: dr.prabhuprakash@gmail.com

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
 For any images presented appropriate consent has been obtained from the subjects. NA
- PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 02, 2019
- Manual Googling: Dec 20, 2019
 iThenticate Software lan 11, 2020 (159)

• iThenticate Software: Jan 11, 2020 (15%)

Date of Submission: Nov 01, 2019 Date of Peer Review: Dec 05, 2019 Date of Acceptance: Dec 26, 2019 Date of Publishing: Feb 01, 2020

ETYMOLOGY: Author Origin