



FUNGAL ETIOLOGY OF OTITIS EXTERNA

Microbiology

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ABSTRACT

Otitis externa (external ear infection) is one of the main causes of hearing loss and can be treated properly after the study of its etiology. Present study conducted to isolate and identify the pathogenic fungi from otitis externa. 500 samples from the infected ear of patients were collected for the isolation of fungi. Total 144 fungi were isolated and six fungi showing different characteristics were identified on the basis of macroscopic, microscopic, and molecular identification was done from NCIM NCL Pune. The fungi were identified as *Aspergillus welwitschiae* (MZ960141), *Aspergillus foetidus* (MZ955454), *Aspergillus tamari* (MZ955985), *Aspergillus aflatoxiformans* (OK035443), *Penicillium polonicum* (ON811480), and *Candida Africana* (ON811599).

KEYWORDS

External ear infection, pathogenic fungi, Otitis externa

INTRODUCTION

The external ear is a micro-environment in itself and harbours various microbes. It is a dockyard of copious bacteria and the health of the external auditory canal is marked by the reciprocity of numerous factors like moisture, pH, cerumen and trauma to skin (Ghanpur *et al.*, 2017). Otitis externa nothing but the inflammation of the external ear counting ear pinna and external auditory canal and may also involve tympanic membrane. It is similar to the infection of skin and soft tissues elsewhere (Enoz & Sevinc, 2009). There are three types of otitis externa, Acute, Chronic and Malignant based on the severity. Acute otitis externa is also known as swimmer's ear and it is diffuse inflammation of the external auditory canal (Hui *et al.*, 2013). Chronic otitis externa is characterized as thickening of the external auditory canal skin secondary to low grade infection and inflammation. It is caused by irritation from drainage through a perforated tympanic membrane. Malignant external otitis infection begins as an inflammation of the external auditory canal that later spreads to cartilage and bone of the skull base. It generally affects diabetics and immunocompromised people (Bhat *et al.*, 2015).

One of the causes of otitis externa is microbial infection includes bacteria and fungi. Bacterial otitis externa produces ear pain or discomfort, otorrhea, pruritus and tenderness, especially on manipulation of the ear. Cellulitis of the pinna and regional lymphadenopathy may be present. In case of otitis externa, *Pseudomonas aeruginosa* is the most common Gram negative bacteria and *Staphylococcus aureus* is the most common Gram positive bacteria (Patton, 2010). The most frequently isolated bacterial species was *Pseudomonas aeruginosa* (Bhat *et al.*, 2015). The next species most frequently isolated were *Staphylococcus aureus*, and other *Staphylococcus* sp. (coagulase negative) (Kalantar E, Mosaei M, Ekrami A, 2006), *Bacillus* sp. *Klebsiella* sp. *Enterobacter* sp. (Enoz & Sevinc, 2009), *Streptococcus* sp., *Microbacterium* sp., *Enterococcus* sp. (Roland & Stroman, 2002). Among fungi, it was found that candida species were the most common cultured fungal agents in acute otitis externa. The other common fungal organisms are *Aspergillus fumigatus*, *A. flavus* and *A. niger*, *Mucor* sp. and *Penicillium* sp. (Enoz & Sevinc, 2009).

The present study deals with the isolation and identification of pathogenic fungi from the patients of otitis externa.

MATERIAL AND METHODS

Different ENT hospitals of Akola City and different villages near Akola city were visited for the collection of samples from otitis externa patients suffering from acute, chronic, eczematous and malignant otitis externa. The ethical approval letter was taken from the college Research Advisory Committee for the collection of samples from otitis externa patients. Total 500 samples were collected from different ENT hospitals according to CLSI (Clinical and Laboratory Standards Institutes) guidelines (Ghanpur *et al.*, 2017). Before collecting the samples the NOC of Doctors were taken and the consent forms from the patients were collected and submitted to the doctors. The scraping,

swab and pus samples were collected in sterilized sample collection tubes with a swab (PW1279 Sterile Hiclean Swab Batch No. 18-1009) containing normal saline as a collection medium for the isolation of pathogenic fungi.

The collected samples were inoculated on the sterilized potato dextrose agar and incubated at 28°C for 5 days for the propagation of fungi. All the fungal isolates were identified based on structural, morphological characteristics, microscopic observation and ITS gene sequencing. For molecular identification, the isolated pathogenic fungi were sent to the NCIM NCL, Dr. Homi Bhabha Road, Pune 411008 Maharashtra.

RESULTS

Total 144 fungi were isolated from the otitis externa patients. Total 6 fungal isolates were labeled as Oti1, Oti2, Oti3, Oti4, Oti5 and Oti6 and identified on the basis of macroscopic, microscopic and ITS (Interspaced Transcribe Sequences) gene sequencing. Following are the characteristics of isolated fungi:

Table-1 Macroscopic And Microscopic Characteristics Of Fungal Isolates

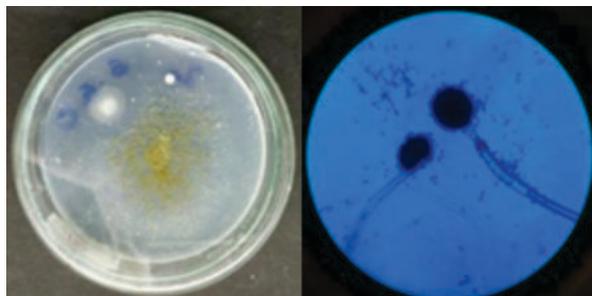
Fungal isolates	Colony colour	Macroscopic features	Microscopic features
Oti1	Black	Radiate heads on basal mycelium	Long conidiophore, long chains of conidia
Oti2	Brownish black	Radiate heads on basal mycelium	Elongated vesicle, long smooth conidia
Oti3	Green	Caramel mustard colony	Rough and warty conidiophore, rough conidia
Oti4	Greenish black	Heads are columnar, both on basal and aerial mycelium	Elongated vesicle, long smooth conidia
Oti5	Brown	Velvety to powdery colonies	Saptate hyphae, branched conidiophore
Oti6	White	Yeast like colonies on PDA	Gram positive yeast with single bud



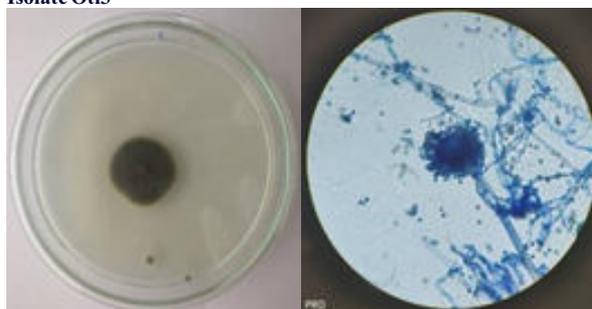
Isolate Oti1



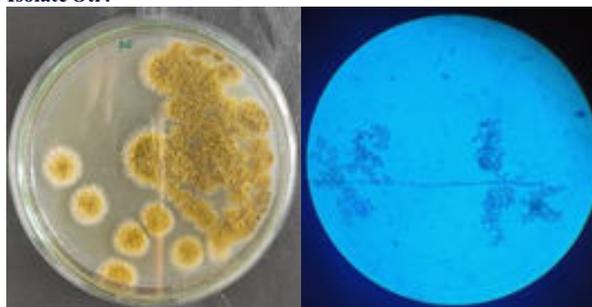
Isolate Oti2



Isolate Oti3



Isolate Oti4



Isolate Oti5



Isolate Oti6

Figure 1 Isolated Pathogenic Fungi On Potato Dextrose Agar And Their Microscopic View

Total 6 fungal isolates were sequenced from NCIMNCL, Pune. Sanger Dideoxy sequencing method was used to sequence the isolated fungi using ITS rRNA gene sequencing. The six isolates were identified as *Aspergillus welwitschiae*, *Aspergillus foetidus*, *Aspergillus tamarii* and *Aspergillus aflatoxiformans*. For phylogenetic tree, the bootstrap method with value 100 was used to draw a tree diagram of neighbor-

joining. The generated molecular data were submitted to NCBI (National Center for Biotechnology Information) which store molecular information about Nucleotides, Proteins and genes. The submitted molecular data is available on www.ncbi.nlm.nih.gov.

Table 2 Identified Pathogenic Fungi With Gene Bank Accession Number

Sr. no.	Code	Name of Fungi	Gene bank Accession
1.	Oti1	<i>Aspergillus welwitschiae</i>	MZ960141
2.	Oti2	<i>Aspergillus foetidus</i>	MZ955454
3.	Oti3	<i>Aspergillus tamarii</i>	MZ955985
4.	Oti4	<i>Aspergillus aflatoxiformans</i>	OK035443
5.	Oti5	<i>Penicillium polonicum</i>	ON811480
6.	Oti6	<i>Candida africana</i>	ON811599

DISCUSSION

Fungal diversity was explored with culture-independent methods by Findley *et al.*, 2013, prepared DNA directly from clinical swabs, PCR-amplified and sequenced two phylogenetic markers within the rRNA region: 18S rRNA and Intervening Internal Transcribed Spacer (ITS) region and generated a custom ITS database based on sequences deposited in Gene Bank to classify sequences to genus-level with greater than 97% accuracy. Hui *et al.*, 2013 cleared some facts about acute otitis externa like its clinical presentation, etiological organisms, management and its prevention. Symptoms include otalgia, itching, fullness and canal pain while chewing. Etiology explained that *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most commonly isolated organisms. Fungal infection is rare but the most common fungi are *Aspergillus* species and *Candida* species. The fungal isolates *Aspergillus*, *Candida albicans* and some dermatophytes were isolated and identified by Vaghela *et al.*, 2016 during the analysis of ear discharge. Nambiar *et al.*, 2015 studied the microbiology of 100 patients with itchy ear and susceptibility profile of the isolated pathogens. Two common aerobic bacteria were isolated *Staphylococcus* (Coagulase positive from 9 patients and Coagulase negative from 22 patients) and *Pseudomonas aeruginosa* from 2 patients. Among fungal isolates *Candida* from 11 patients and from 8 patients *Aspergillus* was isolated.

CONCLUSION

Otitis externa is one of the causes of hearing loss and still get neglected as a small infection. Among various causes of otitis externa microbial infection is main cause includes both bacteria and fungi. Present study revealed that *Aspergillus sp.* dominated the fungal etiology. Not only the bacteria, but also study of fungi is crucial as it can cause severe conditions. The infections, diseases can get proper medications by knowing specific etiology.

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