

## Otomycosis due to *Aspergillus flavus* and *Aspergillus niger* in a flour mill worker

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### Abstract

In this paper, we report an interesting case of dual mycotic infections of the ear in a young male, flour mill worker due to *Aspergillus flavus* and *A.niger* from Gujarat, India. The patient was having pain in his left ear for the last 45 days. Direct microscopical examination of the ear wax by potassium hydroxide technique showed hyaline, septate, thin branched hyphae morphologically simulating to *Aspergillus*. Fungal elements were also observed in the methylene blue stained smears. The culture of the ear swabs and ear wax on Sabouraud dextrose agar with chloramphenicol revealed two filamentous fungi, which on detailed microscopy morphology in Narayan stain confirmed the identity as *Aspergillus flavus* and *A.niger*. Both the fungi were found resistance to clotrimazole and Nystatin but was highly sensitive to mercurochrome. Based on the drug sensitivity test, patient was treated with 2 % solution of mercurochrome for 21 days. The drug showed good clinical response, with no side effects, and hence, it can be recommended for the management of mycotic otitis particularly in poor resource countries, which can hardly afford the expensive antifungal drugs. As Narayan stain is easy to prepare and less expensive, its routine application in microbiology and public health laboratories will help the mycologists to study the detailed morphology of various fungi, which are incriminated in clinical disorders of humans and animals. As far as it could be ascertained, this seems to be first documented record of dual mycotic infection of the ear due to *A.flavus* and *A.niger* in an immunocompetent patient from Gujarat ,India.

KEY WORDS :*Aspergillus flavus*, *Aspergillus niger*, Floor mill worker, Mercurochrome, Narayan stain, Otomycosis

### Introduction

Otomycosis (fungal otitis ,mycotic otitis, mycotic otitis externa) is a global, superficial, sub-acute or chronic fungal infection of the external auditory canal, usually unilateral, and is characterized by inflammation, pain, pruritis, and scaling (Pal,2007).The disease is sporadic in occurrence, and carries no mortality. Fungal otitis is frequently diagnosed in humans, and also in animals, mainly the dogs ( Pal,1982; Nielson, 1985; Pal and Rao,2001; Pal,2007). Mycotic otitis is one of the most commonly encountered clinical disorders in the ear, nose and throat (ENT) clinics (Mygliston and O'Donoghue,1985). The disease is more prevalent in humid and warm climates, particularly during rainy season (Pal, 2007). The disease is worldwide in distribution, and many cases are reported from different regions of India (Pahwa *et al.*,1983; Nielson,1985;Mishra *et al.*,2004; Pal,2007). A large number of fungi, which include moulds and yeasts are implicated in the etiology of mycotic otitis (Jadhav *et al.*, 2003; Mishra *et al.*,2004; Pal,2007). Most of the fungi responsible for otomycosis are ubiquitous in nature, and are frequently recovered from a wide variety of environmental materials (Pal,2008). The fungi usually enter the ear from the environment. Many risk factors, such as antibiotic/steroid therapy, excessive moisture,

abundant cerumen, water irrigation, swimming, concurrent infection, previous trauma, and use of mechanical device to clean/remove of wax are known to predispose the subjects to fungal otitis (Chander *et al.*,1996; Pal,2007). The paucity of literature on dual infection of the ear prompted us to report this unusual case of unilateral otomycosis due to *A.flavus* and *A.niger* in a healthy patient, who was continuously exposed to the fungi in his occupation.

### Materials and Methods

The ear swabs and earwax collected from 27- year-old patient constituted the material for this investigation. The patient who was working in a flour mill visited the skin outpatient (OPD) department for dermatological problem. Later, the patient narrated that he was having pain in his left ear for about 45 days. He did not consult any physician for the ear disorder. The patient was referred to the ear, nose and throat (ENT) OPD for detailed examination. The ear swab and ear wax were collected from both the ears and examined mycologically by direct microscopy in 10 % potassium hydroxide solution (wet mount), smears by methylene blue stain (cytological) as well as cultural examination on Sabouraud dextrose agar with chloramphenicol. The inoculated slants were incubated at 37 C for fungal growth (Pal, 2007). The identification of cultures was made by studying detailed morphology in Narayan stain (Pal, 2004). It contained 6.0 ml of dimethylsulfoxide (DMSO), 4.0 ml of glycerine and 0.5 ml of 3 % aqueous solution of methylene blue. The disc diffusion method was done to test the susceptibility of fungal isolates against clotrimazole, nystatin, and mercurochrome. Topical application of 2 % solution of mercurochrome (three drops two times daily in the affected left ear) was recommended for three weeks.

### Results

The examination of the patient by otoscope helped in tentative diagnosis. The detailed physical and laboratory investigations ruled out the possibility of immunosuppression in our patient. The fungal elements were detected in the earwax of the left ear under potassium hydroxide wet mount preparation and also in the methylene blue stained smears (cytological examination). There was no growth of fungi from the right ear, however, two different fungi were isolated from the affected left ear on Sabouraud medium at 37C. There was no growth of bacteria on nutrient agar. Based on the detailed gross and microscopic morphology in Narayan stain, the isolated fungi were identified as *A.flavus* and *A.niger* (Pal, 2007). Both the fungi showed sensitivity to mercurochrome but were found resistance to clotrimazole, and nystatin. The patient responded well with topical use of mercurochrome, and no side effects of the drug could be observed as per the information of the patient.

### Discussion

Otomycosis, caused by many fungi, is more common in persons who belong to low socio-economic group. The incidence of disease is more between 10 to 40 years of age group (Pal,2007). Our patient was 27- year- old male, and belonged to the poor strata of society. As narrated by the patient, he got his earwax removed by a quack in his village. We believed that the patient would have received minor trauma in his left ear. This probably gave the opportunity for the airborne fungi to enter the ear, and produce the infection. Moreover, the patient was occupationally exposed daily to the fungi, which are widely prevalent in Indian environment (Pal, 2007). As the climate of Gujarat is tropical, we isolated *A.flavus* and *A.niger* from the ear as compared to *Candida albicans*, which is more frequently recovered from temperate zones (Jadhav *et al.*,2003). Mixed infection due to *A.niger* and *C.albicans* is reported in few patients (Pal, 2007). However, in the present case, dual infection of the ear was caused by *A.flavus* and *A.niger*. The efficacy of Narayan stain to study the fungal morphology, and topical use of mercurochrome in the treatment of fungal otitis have been reported by earlier investigators ( Chander *et al.*, 1996; Mishra

*et al.*,2004; Dave and Pal, 2014; Dave *et al.*, 2014). Therefore , we strongly recommend that the wider use of Narayan stain and mercurochrome will certainly be very helpful in the study and management of otomycosis. The persons who work in such environment are advised to use long cap on the head to protect the entry of airborne fungi in the ears, and also not to seek the help of quack for cleaning the ears.

### Conclusion

Otomycosis caused by many fungi is a growing health problem of global significance. Prompt diagnosis and treatment will relieve the suffering of the patient. Cytological examination of ear wax in methylene blue stain is a very simple and inexpensive tool to make the diagnosis of fungal otitis, and hence, it is recommended in rural based clinics/hospitals, which do not have facility for cultural isolation of fungi. In addition, emphasis is given on the wider use of Narayan stain in laboratory for morphological studies of fungi, and topical application of mercurochrome solution for the treatment of mycotic otitis.

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