

Epidemiological characteristics of otomycosis in Tehran, Iran: A study with emphasis on molecular identification and antifungal susceptibility of species

within *Aspergillus* section *Nigri*

Zahra kamali sarvestani¹, Roshanak DaieGhazvini¹, Shahram Mahmoudi¹, Kazem Ahmadikia¹, S.jamal Hashemi¹, Amirhosein Davari².

1.Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences(TUMS), Tehran, Iran
2.Department of Molecular and Medical Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Objective: Otomycosis is a superficial infection of the ear caused by a spectrum of various fungal agents in particular species under *Aspergillus* and *Candida* genera. Black aspergilli (section *Nigri*), particularly *Aspergillus niger* is the most prevailing causative agents of otomycosis. However, using morphological criteria alone, discrimination of species within section *Nigri* -A number of different species whose morphological features resemble those of *A. niger*- cannot be reliably achieved. Due to different susceptibility patterns among species under section *Nigri* to antifungal agents and appropriate treatment, species delimitation of this section is issue of great importance. The aim of this study was to determine the frequency of otomycosis in Tehran, Iran, with emphasis on molecular identification and determination the susceptibility pattern of a set of black aspergilli isolated from otomycosis patients.

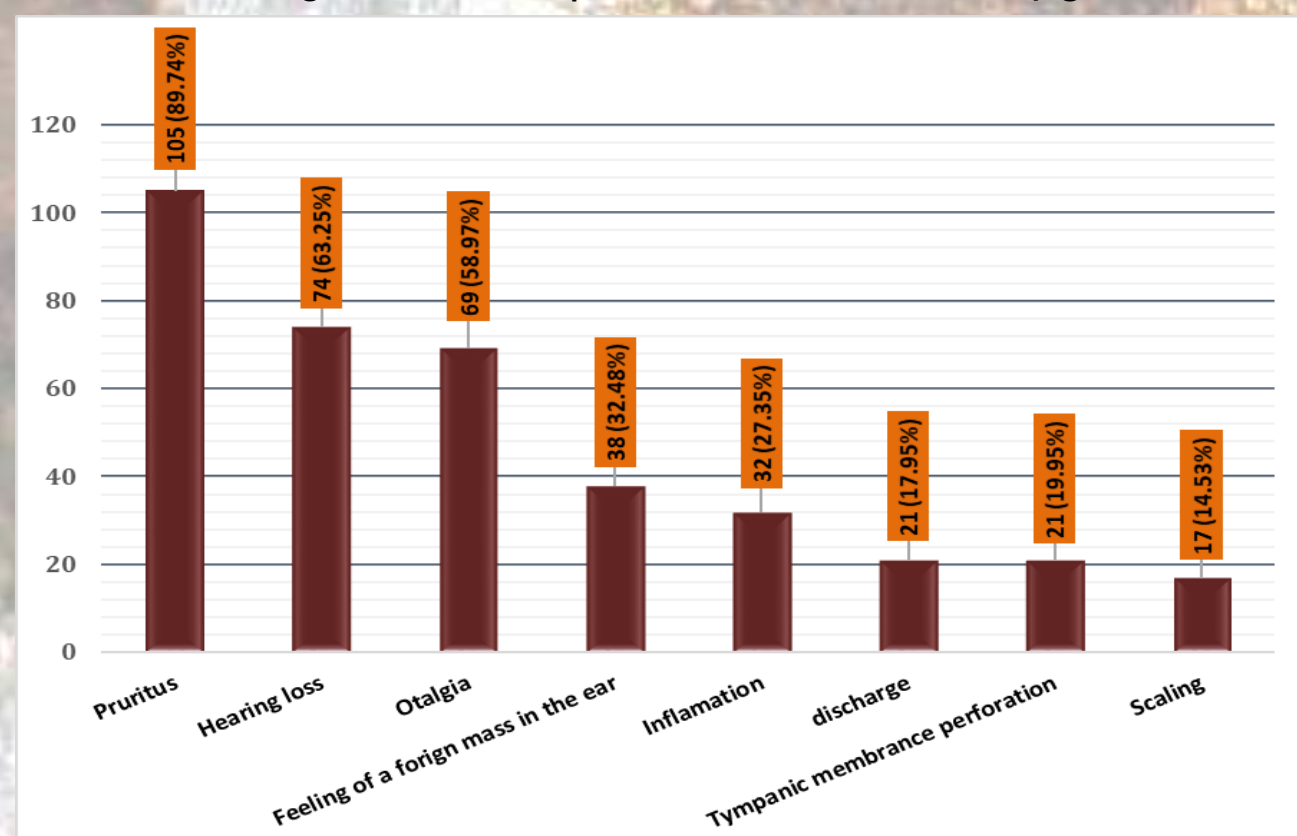
Methods: From Apr 2016 to Jan 2017 a set of 412 subjects with a suspicion of external otitis were included. Clinical examination and specimen collection was performed by an otorhinolaryngologist. Subsequently, direct examination and culture was performed on specimens and isolated molds were identified morphologically. Yeast isolates were identified using CHROMagar candida medium and PCR-RFLP of ribosomal DNA whenever needed. Black *Aspergillus* isolates from otomycosis patients were identified by using the PCR-sequencing of the β -tubulin gene. Furthermore, the susceptibility of black aspergilli isolates to three antifungal drugs, including fluconazole (FLU), clotrimazole (CLT), and nystatin (NS), were examined according to CLSI M38-A2.

Results: A total of 117/412 (28.4%) included patients were diagnosed with otomycosis including 64 (54.7%) males and 53 (45.3%) females. Patients were within the age range of 10-75 years and the highest prevalence was found in the age group of 46-55 years (30.77%). Pruritus (89.74%) and auditory manipulation/trauma (83.76%) were the predominant symptom and predisposing factor, respectively. From 117 patients 126 isolates were recovered, black aspergilli (n=43, 34.1%) were the most common etiologic agents and *Candida glabrata* (n=25, 20%) was the predominant isolated yeast. Furthermore, 16 cases of mixed otomycosis were identified and coinfection due to *A. niger* and *C. glabrata* (seven cases) were the predominant pattern. While, with sequence-based methods the majority of black aspergilli isolates were identified as *A. tubingensis* (32/43, 74.42%) followed by *A. niger* (11/43, 25.58%).

The different patterns of mixed fungal otitis due to *Aspergillus* and *Candida* species observed among 117 patients with otomycosis

Fungal agents	number of patients
<i>A. niger</i> + <i>C. glabrata</i>	7
<i>A. niger</i> + <i>A. flavus</i>	4
<i>A. flavus</i> + <i>C. glabrata</i>	2
<i>A. niger</i> + <i>C. parapsilosis</i>	1
<i>A. flavus</i> + <i>C. albicans</i>	1
<i>A. flavus</i> + <i>C. tropicalis</i>	1
Total	16

The lowest minimum inhibitory concentration (MIC) values were observed for NS with geometric means (GM) of 4.65 μ g/ml and 4.83 μ g/ml against *A. tubingensis* and *A. niger* isolates, respectively. CLT showed wide MIC ranges and a statistically significant inter-species difference was observed between *A. tubingensis* and *A. niger* isolates ($p < 0.05$). FLU was inactive against both species with GMs > 64 μ g/ml.



The frequency of different clinical symptoms among 117 patients with otomycosis

The minimum inhibitory concentration (MIC) values of three antifungal drugs against *Aspergillus* section *Nigri* isolated from otomycosis patients

Species (N=43)	Antifungal drugs	MIC values (μ g/ml)							Range	MIC50	MIC90	GM	
		2	4	8	16	>16	3	64					>64
<i>A. tubingensis</i> (32)	FLU							2	30	64 - >64	>64	>64	>64
	CLT		5	14	12	1				4 - >16	8	16	9.7
	NS	1	23	8						2 - 8	4	8	4.6
<i>A. niger</i> (11)	FLU								11	>64	>64	>64	>64
	CLT	1	6	3	1					2 - 16	4	8	5.1
	NS		8	3						4 - 8	4	8	4.8

Conclusion: Species other than *A. niger* can be more frequent as observed in our study. In addition, considering the low and variable activity of tested antifungal drugs, empirical treatment can result in treatment failure. Accurate identification and antifungal susceptibility testing of isolates is, however, recommended.

KEYWORDS: *Aspergillus niger*; *Aspergillus tubingensis*; Iran ; section nigri; Otomycosis; antifungal agents