Original Article

Lamisil, a potent alternative antifungal drug for otomycosis

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Abstract

Background and Purpose: Otomycosis is an acute, subacute or chronic fungal infection of the pinna, the external auditory meatus and the ear canal caused mainly by several species of saprophytic fungi. Lamisil (Terbinafine) is an allylamine antifungal agent, that is used both in the topical and oral administration for the treatment of dermatophytosis, cutaneous candidiasis, and the pityriasis versicolor. We investigated the *in vitro* activity of clotrimazole, miconazole, nystatin, and Lamisil against the agents of otomycosis.

Materials and Methods: Fifteen clinically obtained isolates from otomycosis (*Aspergillus* species; n=13, and *Candida* species, n=2) and 8 environmental isolates of *Aspergillus* were tested. The disk diffusion method was employed to detect susceptibility. In the present study, the *in vitro* activity of the terbinafine with clotrimazole, miconazole, and nystatin against several isolates of *Aspergillus* and *Candida* with different sources were compared.

Results: Out of 23 isolates of *Aspergillus*, *Candida* 4(17.4%) and 1(4.4%) were resistant to nystatin and miconazole, respectively. In addition, all tested organisms were sensitive to clotrimazole and terbinafine. Statistical analysis has shown that there are no significant differences on the effects of clotrimazole, miconazole and, terbinafine on saprophytic (environmental) and pathogenic isolates of *A. niger*, *A. flavus*, and *A. terreus* (P value= 0.85). In addition, all tested organisms were found to be highly susceptible to terbinafine (P< 0.04).

Conclusion: This is a new approach for the possible use of Lamisil for the treatment of otomycosis.

Keywords: *Aspergillus* species, *Candida* species, Lamisil, Otomycosis

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Introduction

Otomycosis is an acute chronic fungal infection of external auditory meatus and the ear canal that is caused mainly by several species of saprophytic fungi [1]. A disease has a worldwide distribution with a higher prevalence in the tropical and subtropical regions. Dusty, humid, and warm conditions favor for otomycosis. The latter disorder is a secondary infection that usually occurs with following bacterial infection in the ear canal. The most common bacteria, such as *Pseudomonas* and *Proteus* species and *Staphylococcus aureus* are co-infections in such a disease. A disease is usually caused by the saprophytic fungi, especially *Aspergillus niger* followed by *A. flavus*, *A. terreus*, *A. fumigatus*, *Pseudallescheria boydii*, *Scopulariopsis* species, and *Candida* species [1-6], although *dermatophytes* and *Malassezia* species have fewer roles in the disease [7]. The predisposing factors for the disease are the presence of the cerumen, instrumentation of ear (hearing aids, foreign body, and cleaner abusers), poor hygiene, abusers of oils, steroid therapy, and swimming, especially in the contaminated water.

The usual treatment protocol for otomycosis is applying the topical antifungal agent along with the cleaning of the external ear canal. Clotrimazole, miconazole, and nystatin are available as the topical antifungal for the treatment of otomycosis. Lamisil (Terbinafine) is an allylamine antifungal agent that, is used both in the topical and oral administration for the treatment of dermatophytosis, cutaneous candidiasis, pityriasis versicolor and so on [8]. It is a safe antifungal for the topical or the systemic treatment. Several reports show that Lamisil has potent activities against the saprophytic fungi, viz, *Aspergillus* species [8, 9]. However; few reports describe the

antifungal effect of Lamisil against the agents of otomycosis such as *Aspergillus*, *Candida* and others[8].

The aim of the present study was to evaluate the *in vitro* activities of clotrimazole, miconazole, and nystatin against *Aspergillus* and *Candida* species obtained from the patients suffering from otomycosis as well as the environmental strains. In addition, the sensitivity of agents towards the above antifungal was compared to lamisil.

Material and Methods

Isolates and identification

Fifteen isolates were obtained from otomycosis and eight isolates were collected from the routine culture contamination. They included 21 isolates of *Aspergillus*, (8 isolates from the environment and 13 isolates from otomycosis) and two isolates of *Candia glabrata* from otomycosis. All *Aspergillus* isolates were identified by the standard methods; the macroscopic and microscopic features cultured on Sabouraud's dextrose agar, SDA (Merck, Germany). Yeasts were also detected with CHROMagar® Candida medium (CHROMagar® Candida Company, Paris, France), germ tube production on fresh serum at 37°C and morphology on Cornmeal agar (Difco, USA). Isolates were stored as suspensions in the sterile water at 4°C until used.

Antifungal agents

The disks of clotrimazole, miconazole, and nystatin were obtained (Liofilchem Bacterriology Products, Italy) in the potence of 50µg/disk, 10µg/disk, and 100 U/disk, respectively. The Lamisil antifungal drug supplied by the manufacturer as powder was used (Tehran Chimi Co., Tehran, Iran.). The stock solution (10%) of terbinafine was prepared with dimethyl sulfoxide (DMSO). The Lamisil disks were prepared considering the potence, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 12µg/disk. The disks were dried at ambient temperature for several hours and then stored at -20°C until used.

In vitro antifungal susceptibility testing

The isolates were subcultured on SDA and incubated at the ambient temperature for 48 h. The suspension culture (yeasts, *Aspergillus* spores) was prepared in sterile PBS and adjusted to a concentration of 10^6 CFU/ml. The sterile swab was dipped into the fungal suspension then rolled on the surface of the agar medium [10]. The inoculated plates were dried for 15 min at room temperature in the laminar hood. Then clotrimazole, miconazole, nystatin, and terbinafine disks were applied to the inoculated agar with forceps. The plates were incubated at ambient temperature for 24-48 h and then bioactivities were determined by measuring the diameter of inhibition zone diameter in mm (Figure1). The zone diameters (mm) for all antifungal disks at 24 h were measured. For all anti-fungal drugs, the presence of a clear and visible zone (mm) was measured with no colonies inside them[11].

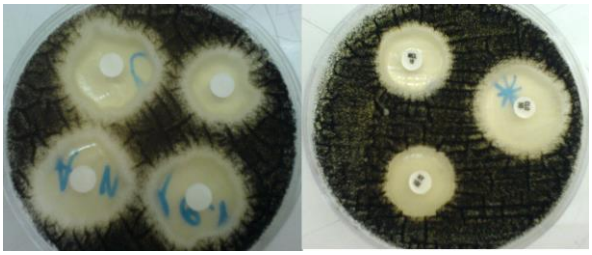

Figure 1. Diameter of inhibition zone in mm around the Lamisil disks (Different concentrations) (Left), clotrimazole, miconazole, and nystatin (right)

Results and Discussion

Fifteen clinical isolates of *Aspergillus* and *Candida* and eight environmental isolates of *Aspergillus* were studied. The disk diffusion testing of clotrimazole, miconazole, nystatin and lamisil on the isolates was performed. The disk diffusion is a rapid method for testing

Table 1. The susceptibility of otomycosis agents to clotrimazole, miconazole, and nystatin

Species	Inhibition zone (mm)		
	Nystatin (100U)	Miconazole 10µg/disk	Clotrimazole 50µg/disk
A. niger (6)			
Range	20-23	22-27	30-34
Mean	21.3	24.0	32.0
A. terreus (2)			
Range	13-15	28-36	28-40
Mean	14.0	32.0	34.0
C. glabrata (2)			
Range	20	30	38-40
Mean	20.0	30.0	39.0
A. flavus (5)			
Range	18-20	16-30	34-42
Mean	20.6	23.0	36.8

Table 2. The susceptibility of otomycosis agents to Lamisil

Species	Inhibition zone (mm), Lamisil (µg/disk)							
	0.125	0.25	0.5	1	2	4	8	12
A. niger (6)								
Range	9-20	11-22	12-22	17-28	24-34	26-34	26-36	26-36
Mean	13.7	16.0	16.3	22.0	27.7	29.7	29.8	31.0
A. terreus (2)								
Range	18-20	20-22	20-22	26-30	30-34	30-36	34-40	34-40
Mean	19.0	21.0	21.0	28.0	32.0	33.0	37.0	37.0
C. glabrata (2)								
Range	0	10-11	12	14-20	25	26-27	27-28	30
Mean	0.0	10.45	12.0	17.0	25.0	26.5	27.5	30.0
A. flavus (5)								
Range	17-32	19-26	20-38	23-40	34-40	34-50	34-50	34-50
Mean	22.6	22.6	27.6	29.4	36.0	40.0	40.2	42.4

antifungal and offers an attractive alternative for testing rapidly [10, 12]. The range and mean inhibition zone of clotrimazole, miconazole and nystatin for all otomycosis agents are shown in Table 1. In addition, the Minimum inhibitory concentration (MICs), the range, and the mean inhibition zone of Lamisil for all otomycosis agents are shown in Table 2. As shown, all tested organisms are more sensitive to Lamisil than other drugs. For example, the inhibition zone of Lamisil (12 µg/disk) for *A. niger* is 31 mm compared to 32 mm for clotrimazole (50 µg/disk).

We carried out an *in vitro* study of the susceptibility of *Aspergillus* and *Candida* species obtained from otomycosis and we observed a potent activity of Lamisil compared to clotrimazole, miconazole, and nystatin. We did not measured the MICs of tested antifungals for isolates; however, the sensitivity of isolates to lamisil is more valuable than clotrimazole, miconazole, and nystatin. In the literature, only two studies were found in which the in vitro activity of terbinafine against *Aspergillus* species isolated from otomycosis was tested [8, 13]. Karaarslan et al., [8] have shown that both itraconazole and terbinafine showed an *in vitro* activity against otomycosis agents. In the present study, the environmental isolates of *A. niger*, *A. flavus*, and *A. terreus* were also tested against clotrimazole, miconazole, nystatin, and terbinafine (Tables 3, 4). As shown, all isolates are more susceptible to terbinafine than other antifungals.

Table3. The susceptibility of the environmental species to nystatin, miconazole and clotrimazole

Species	Inhibition zone (mm)		
	Nystatin (100U)	Miconazole 10µg/disk	Clotrimazole 50µg/disk
A. niger (4)			
Range	20-21	21-25	30-32
Mean	20.3	24.0	31.0
A. terreus (2)			
Range	15-16	30-32	40-42
Mean	15.5	31.0	41.0
A. flavus (2)			
Range	18-20	26	35-36
Mean	19.6	26.0	35.5

In conclusion, our finding suggested that Lamisil might be useful in the treatment of the otomycosis infection caused by *Aspergillus* and *Candida*.

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Table4. The Susceptibility of the environmental species to Lamisil

Species	Inhibition zone (mm), Lamisil (µg/disk)							
	0.125	0.25	0.5	1	2	4	8	12
A. niger (4)								
Range	11-18	13-18	14-19	17-22	22-28	23-30	24-30	24-32
Mean	14.0	16.0	16.3	19.0	24.8	28.3	27.5	29.0
A. terreus (2)								
Range	15-20	11-22	21-23	24-26	32	34-40	38	40-42
Mean	17.5	16.5	22.0	25.0	32.0	37.0	38.0	41.0
A. flavus (2)								
Range	20	14-18	22-24	27-31	38-40	40-42	40-42	42-44
Mean	20.0	16.0	23.0	29.0	39.0	41.0	41.0	43.0

Authors' contributions

A.ZM. designed andmanaged the research, wrote manuscript and edited the final manuscript. Z.S. applied all tests and M.G. analyzed data.

Conflicts of interest

The authors state no conflict of interest.

Financial Disclosure

No financial interests related to the material of this manuscript have been declared.

References

1. Zarei Mahmoudabadi A, Masoomi SA, Mohammadi H. Clinical and mycological studies of otomycosis. Pak J Med Sci. 2010; 26(1):187-90.
2. Nemati S, Hassanzadeh R, Khajeh Jahromi S, Delkhosh Nasrollah Abadi A. otomycosis in the north of Iran: common pathogens and resistance to antifungal agents. Eur Arch of Otorhinolaryngol. 2014; 271(5): 953-7.
3. Barati B, Okhovvat SA, Goljanian A, Omrani MR. otomycosis in central iran: a clinical and mycological study. Iran Red Crescent Med J. 2011;13(12): 873-6.
4. Mogadam AY, Asadi MA, Dehghani R, Hooshyar H. The prevalence of otomycosis in Kashan, Iran, during 2001-2003. Jundishapur J Microbiol. 2009; 2(1): 18-21.
5. Baumgartner BJ, Rakita RM, Backous DD. *Scedosporium apiospermum* otomycosis. Am J Otolaryngol. 2007; 28(4): 254-6.
6. Saki N, Rafiei A, Nikakhlagh S, Amirrajab N, Saki S. Prevalence of otomycosis in Khouzestan Province, south-west Iran. J Laryngol Otol. 2013; 127(1): 25-7.
7. Degerli K, Ecemis T, Gunhan K, Baskesen T, Kal E. Agents of otomycosis in Manisa region, Turkey, 1995-2011. Mikrobiyol bul. 2012; 46(1): 79-84.
8. Karaarslan A, Arikan S, Ozcan M, Ozcan KM. In vitro activity of terbinafine and itraconazole against *Aspergillus* species isolated from otomycosis. Mycoses. 2004; 47(7): 284-7.
9. Mosquera J, Sharp A, Moore CB, Warn PA, Denning DW. In vitro interaction of terbinafine with itraconazole, fluconazole, amphotericin B and 5-flucytosine against *Aspergillus* spp. J Antimicrob Chemother. 2002; 50(2): 189-94.
10. Klevay M, Ebinger A, Diekema D, Messer S, Hollis R, Pfaller M. Disk diffusion testing using *Candida* sp. colonies taken directly from CHROMagar Candida medium may decrease time required to obtain results. J Clin Microbiol. 2005; 43(7): 3497-9.
11. Espinel-Ingroff A, Canton E, Fothergill A, Ghannoum M, Johnson J, Jones RN, et al. Quality control guidelines for amphotericin b, itraconazole, posaconazole, and voriconazole disk diffusion susceptibility tests with nonsupplemented mueller-hinton agar (clsi m51-a document) for nondermatophyte filamentous fungi. J Clin Microbiol. 2011; 49(7): 2568-71.
12. Espinel Ingroff A, Arthington Skaggs B, Iqbal N, Ellis D, Pfaller MA, Messer S, et al. Multicenter evaluation of a new disk agar diffusion method for susceptibility testing of filamentous fungi with voriconazole, posaconazole, itraconazole, amphotericin B, and caspofungin. J Clin Microbiol. 2007; 45(6): 1811-20.
13. Rotoli M, Sascaro G, Cavalieri S. *Aspergillus* versicolor infection of the external auditory canal successfully treated with terbinafine. Dermatol. 2001; 202(2): 143.