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 *Candida 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 Bacteriology 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)](image)

**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

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

| Species          | inhibition zone (mm) |
|------------------|-----------------------|
|                  | Nystatin (100U)  | Miconazole 10µg/disk | Clotrimazole 50µg/disk |
| *A. niger* (6)   | 20-23               | 22-27                 | 30-34                  |
| Range            | 21.3                | 24.0                  | 32.0                   |
| Mean             |                      |                      |                        |
| *A. terreus* (2) | 13-15               | 28-36                 | 28-40                  |
| Range            | 14.0                | 32.0                  | 34.0                   |
| Mean             |                      |                      |                        |
| *C. glabrata* (2)| 20                  | 30                    | 38-40                  |
| Range            | 20.0                | 30.0                  | 39.0                   |
| Mean             |                      |                      |                        |
| *A. flavus* (5)  | 18-20               | 16-30                 | 34-42                  |
| Range            | 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| 31.0 |
| Mean             | 13.7                                   | 16.0  | 16.3 | 22.0 | 27.7 | 29.7 | 29.8 | 31.0 |     |
| A. terreus (2)   | 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)  | 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)    | 17-32                                  | 19-26 | 20-38| 23-40| 34-40| 34-50| 34-50| 34-50|     |
| Mean             | 22.6                                   | 22.6  | 27.6 | 29   | 36   | 40   | 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.

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

Table 3. The susceptibility of the environmental species to nystatin, miconazole and clotrimazole

| Species          | 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)   | 15-16           | 30-32                | 40-42                |
| Range            | 15.5            | 31.0                 | 41.0                 |
| A. flavus (2)    | 18-20           | 26                   | 35-36                |
| Range            | 19.6            | 26.0                 | 35.5                 |

Table 4. 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| 29.0 |
| Mean             | 14.0                                    | 16.0  | 16.3 | 19.0 | 24.8 | 28.3 | 27.5 | 29.0 |     |
| A. terreus (2)   | 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)    | 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 |     |

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Authors’ contributions
A.ZM. designed and managed 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.

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