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In Vitro Antifungal Activity of Luliconazole, Efinaconazole, and Nine Comparators Against *Aspergillus* and *Candida* Strains Isolated from Otomycosis

Gholamreza Shokoohi [1, 2], Reza Rouhi Sefidmazgi [2, 3], Mohammad Etehadnezhad [1, 2], Bahram Ahmadi [4], Javad Javidnia [5, 6], Sadegh Nouripour-Sisakht [7], Farhang Hooshmand [2, 8], Ali Rezaei-Matehkolaei [9], Seyede Nadia Tabatabaeifar [10] and Saham Ansari [11, *]

[1]Department of Medical Parasitology and Mycology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran
[2]Zoonosis Research Center, Jahrom University of Medical Sciences, Jahrom, Iran
[3]Department of Medical Otorhinolaryngology, Jahrom University of Medical Sciences, Jahrom, Iran
[4]Department of Medical Laboratory Sciences, Faculty of Paramedical, Bushehr University of Medical Sciences, Bushehr, Iran
[5]Department of Medical Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[6]Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[7]Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[8]Department of Pathology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran
[9]Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[10]Department of Internal Medicine, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran
[11]Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: samansari1988@yahoo.com

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Abstract

Background: *Aspergillus* and *Candida* species are the most commonly identified fungal pathogens in otomycosis. However, we usually encounter some difficulties in its treatment because many patients show resistance to antifungal agents and present a high recurrence rate.
Objectives: The current research was conducted to compare the *in vitro* activities of luliconazole (LUL), and efinaconazole (EFN) and the nine comparators on *Aspergillus* and *Candida* strains isolated from otomycosis.
Methods: The *in vitro* activities of nine common antifungal drugs (amphotericin B (AMB), voriconazole (VRC), fluconazole (FLU), itraconazole (ITC), ketoconazole (KTO), clotrimazole (CLO), nystatin (NYS), terbinafine (TRB), and caspofungin (CAS)) and two novel new azoles (LUL and EFN) against 108 clinical isolates of *Aspergillus* and *Candida* species obtained from otomycosis were assessed according to the CLSI broth microdilution document.
Results: The LUL and EFN had the geometric mean minimum inhibitory concentrations (GM MICs) of 0.098 and 0.109 μg/mL against all *Aspergillus* strains, respectively. Furthermore, the GM MICs of all *Candida* isolates for LUL, EFN, CAS, CLO, VRC, AMB, ITC, KTO, FLU, NYS, and TRB were calculated to be 0.133, 0.144, 0.194, 0.219, 0.475, 0.537, 0.655, 1.277, 4.905, 9.372, and 13.592 μg/mL, respectively. Additionally, 6 (35.29%), 2 (11.7%), and 1 (5.88%) *Candida* isolates were resistant to FLU, CAS, and VRC, respectively.
Conclusions: As the findings indicated, LUL and EFN showed the lowest GM MIC values against the examined species. Accordingly, these novel imidazole and triazole antifungal agents can be regarded as proper candidates for the treatment of otomycosis caused by *Aspergillus* and *Candida* strains.

Keywords: Otomycosis, Antifungal Susceptibility, Efinaconazole, Luliconazole

1. Background

Otomycosis, a superficial infection of the external ear induced by fungal pathogens, is a common condition in tropical and subtropical regions (1). The similarity of the clinical symptoms of otomycosis with those of other ear infections that cause the improper administration of antifungal drugs leads to resistance to antifungal drugs (2). Some *Aspergillus* and *Candida* species, which are the main causes of otomycosis, have an inherent resistance to several antifungal drugs (3). There is evidence regarding the isolation of the multidrug-resistant *Candida* species, namely *Candida auris*, one of the most threatening fungal species, from an external ear discharge (4).

Topical forms of clotrimazole, miconazole, bifonazole, ciclopirox olamine, tolnaftate, and amphotericin B, as well as triazoles, such as voriconazole, posaconazole, and itraconazole for oral administration are instances of accessible therapies for otomycosis (5, 6). Although the causative agents of otomycosis are commonly susceptible to most antifungal drugs *in vivo* and *in vitro*, treatment is a big challenge because frequent relapses and failures are observed, particularly in cases with complete removal of these organisms, which can act as a potential risk factor leading to the gradual increase of resistant species (7, 8). Thus, alternative antifungal agents with better activity and lower side effects can help to improve the controlling of these infections. Lately, the new imidazole agents, luliconazole and efinaconazole, were approved for the treatment of onychomycosis and dermatophytosis (9). Previous studies have also shown the potent activities of these antifungal agents against different strains of fungi (10-12).

2. Objectives

As far as we know, only limited data are available on the *in vitro* activities of these novel imidazoles, and triazoles against *Aspergillus* and *Candida* strains isolated from otomycosis (13). Therefore, the current research was performed to compare the *in vitro* activities of two novel antifungal agents, namely luliconazole (LUL) and efinaconazole (EFN), with those of amphotericin B (AMB), voriconazole (VRC), fluconazole (FLU), itraconazole (ITC), ketoconazole (KTO), clotrimazole (CLO), nystatin (NYS), terbinafine (TRB), and caspofungin (CAS) against *Aspergillus* and *Candida* strains isolated from otomycosis.

3. Methods

3.1. Fungal Isolates

A total of 91 clinical *Aspergillus* strains (*Aspergillus tubingensis* [n = 57], *A. niger* [n = 28], *A. flavus* [n = 5], and *A. awamori* [n = 1]) and 17 *Candida* strains (*C. parapsilosis* [n = 7], *C. glabrata* [n = 6], *C. albicans* [n = 3], and *C. orthopsilosis* [n = 1]) obtained from patients with otomycosis, in Jahrom, Iran. The genomic DNA of these fungal strains, previously extracted by the glass-bead phenol-chloroform (6). *Candida* isolates were identified at the species level based on molecular methods to obtain restriction digestion of the internal transcribed spacer rDNA region using the PCR-restriction fragment length polymorphism (PCR-RFLP) method with the MspI restriction enzyme (14). The identification of the *Aspergillus* isolates was also accomplished by the amplification and sequencing of a fragment of the β-tubulin gene using beta-2A and beta-2B primers, respectively (15). Randomly selected isolates were reconfirmed by DNA sequencing of the ITS rDNA and β-tubulin regions. The obtained nucleotide sequences were deposited in GenBank under the accession numbers: MT348163- MT348208 and MT303056-MT303061.

3.2. In Vitro Antifungal Susceptibility Test

In vitro antifungal susceptibility of the yeast strains and mold isolates were tested based on the Clinical and Laboratory Standards Institute (CLSI) M27-A3/M60 and M38-A3, respectively (16-19). After the dilution of the antifungal drugs in the standard RPMI-1640 medium (Sigma, St. Louis, MO, USA.) with L-glutamine and without bicarbonate, they were buffered by 0.165 M-morpholinepropanesulfonic acid (MOPS) buffer (Sigma, St. Louis, MO, USA) and using NaOH, the acidity was adjusted to pH 7.0. Drug serial dilutions were then dispensed into 96-well microdilution trays at a concentration range of 0.016 - 16 μg/mL for ITC (Janssen Research Foundation, Beerse, Belgium), VRC (Pfizer, Central Research, Sandwich, United Kingdom), CLO (Sigma, St. Louis, MO, USA), KTO (Sigma, St. Louis, MO, USA), TRB (Sigma, St. Louis, MO, USA), CAS (Sigma, St. Louis, MO, USA), and AMB (Bristol-Myers-Squib, Woerden, The Netherlands). This concentration range was determined as 0.063 - 64 μg/mL for FLU (Pfizer) and NYS (Sigma, St. Louis, MO, USA) and 0.001 - 1 μg/mL for LUL (Sigma, St. Louis, MO, USA, and EFN (Sigma, St. Louis, MO, USA).

The spectrophotometric adjustment of homogeneous freshly yeast or conidial suspensions that cultured on Sabouraud dextrose agar containing chloramphenicol (50 mg/L) (SDA, Difco Laboratories, Detroit, MI, USA) was accomplished at a wavelength of 530 nm to the optical densities (ODs) of 75 - 77% and 80 - 82% for yeasts and molds, respectively. The final inocula suspension was adjusted to 0.5 - 2.5 $\times 10^3$ and 0.4 - 5 $\times 10^4$ conidia/mL for yeasts and molds, respectively, in RPMI-1640 with L-glutamine and without sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA) and buffered at a pH 7.0 with 0.165 MOPS (Sigma-Aldrich, St. Louis, MO, USA).

Based on CLSI guidelines (CLSI M27-A3 and M27-S4 documents), *Candida* species were grown at 35°C, and microdilution plates were incubated at 35°C for 24h (if no growth was observed or growth was inadequate, incubation was extended to 48 h) and read visually (16). For *Aspergillus* species, plates were incubated at 35°C for 24 - 72h (18). After the incubation of the microdilution plates, they were visually examined to determine the minimum inhibitory concentration (MIC) values. *Candida krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019), and *Paecilomyces variotii* (ATCC 22319) were utilized as quality controls for each new series of MIC plates.

The determination of the MIC values of the yeasts and molds was performed based on the guidelines of the CLSI M27-A3/M60 and M38-A3, respectively (16-18). The geometric mean (GM) MICs, MIC_{50}, MIC_{90}, and MIC ranges were estimated for all antifungals. Susceptibility and resistance to antifungal drugs for *Candida* species isolates was evaluated based on CLSI-S4 and MIC value of various *Aspergillus* species and compared with epidemiological cutoff values (ECV) for *in vitro* susceptibility testing using the CLSI-M59 method (19). All tests were carried out in duplicate.

3.3. Statistical Analysis

The student's *t*-test was utilized to determine the differences in the mean values using SPSS software (version 16.0) at a significance level of ≤ 0.05.

4. Results

Tables 1 and 2 present the GM MIC, MIC range, MIC_{50}, and when appropriate MIC_{90} of the studied antifungals. The LUL and EFN had the GM MICs of 0.098 and 0.109 μg/mL against all *Aspergillus* strains, respectively. These values were obtained to be 0.377, 0.777, 3.568, 3.695, 4.922, 8.311, 8.503, 0.146, and 13.739 μg/mL for VRC, CLO, ITC, FLU, AMB, KTO, CAS, TRB, and NYS, respectively. The comparison of the susceptibilities of the strains revealed a high MIC value for *A. niger* in all drugs, except for LUL, EFN, and TRB, which had low MICs. With regard to the *Candida* isolates, the GM MICs LUL, EFN, CAS, CLO, VRC, AMB, ITC, KTO, FLU, NYS, and TRB were calculated to be 0.133, 0.144, 194, 0.219, 0.475, 0.537, 0.655, 1.277, 4.905, 9.372, and 13.592 μg/mL, respectively (Table 2). A total of 7 (41.1%) and 4 (23.5%) *Candida* isolates were found to be susceptible-dose-dependent to FLU and VRC, respectively. Additionally, 6 (35.29%), 2 (11.7%), and 1 (5.88%) *Candida* isolates were resistant to FLU, CAS, and VRC, respectively (Table 2).

5. Discussion

The present research assessed the *in vitro* susceptibility testing of 108 molecularly well-characterized *Aspergillus*, and *Candida* isolates recovered from otomycosis patients to LUL, a novel imidazole, and EFN, a novel triazole, and comparing them with other antifungal drugs. The LUL and EFN are new imidazole and triazole agents, which their efficacy in the management of dermatophytosis and onychomycosis has been recently confirmed. The literature is indicative of the potent activities of these antifungals against dermatophytes, *C. albicans*, dematiaceous fungi, *Malassezia*, and azole-resistant and azole-susceptible *A. fumigatus* strains (7, 12, 20-22). However, to the best of our knowledge, there is no report regarding the resistance of these species to these drugs. As the results of the current research indicated, LUL (0.098 μg/mL) and EFN (0.109 μg/mL) had the lowest GM MICs against *Aspergillus* strains. The MIC ranges of LUL against *A. niger* and *A. tubingensis* isolates were 0.06 - 0.25 and 0.031 - 0.5 μg/mL, respectively. These values for EFN were 0.06 - 0.5 and 0.06 - 0.5 μg/mL, respectively (Table 1).

In a study performed by Hivary et al., the MICs of LUL against *A. niger* and *A. tubingensis*, as the predominant agents of otomycosis, were in the ranges of 0.00024 - 0.125 μg/mL and 0.00024 - 0.125 μg/mL, respectively (20). In addition, the MIC value presented by Tupaki-Sreepurna et al. demonstrated that EFN was more potent than other azoles against different non-dermatophytic fungi (21). The EFN MIC values was estimated at < 0.25 μg/mL for *Aspergillus* (12, 21, 23). Our results revealed low MIC values for EFN and LUL against *Aspergillus*, which is in line with other reports (12, 21, 23). In the current research, TRB had the GM MICs of 0.040, 0.128, and 0.179 μg/mL against *A. flavus*, *A. niger*, and *A. tubingensis*, respectively. The TRB (Lamisil) is a safe allylamine antifungal used both topically and orally for the management of infections, such as pityriasis versicolor, cutaneous candidiasis, and dermatophytosis (24). Based on a study by Mosquera et al., this drug had potent activities against *Aspergillus* species (25). In the present study, TRB had a lower GM MIC against *A. flavus* and *A. niger* compared to the values reported by Moore et al. (26). The VRC and CLO are other antifungal agents utilized for the treatment of *Aspergillus*-induced infections.

The susceptibility of *Aspergillus* species to these antifungals was also investigated in the present study. The comparison of VRC and CLO with ITC, FLU, KTO, and AMB revealed a lower GM MIC for VRC and CLO against *Aspergillus* species. The results of previous studies are indicative of the resistance of this species to VRC (27). Sutton et al. suggested VRC as an agent that can be used clinically for the treatment of refractory invasive aspergillosis and should be prioritized over the conventional AMB treatment (28). There are reports regarding the growing resistance of ITC to *A. fumigatus* (29). On the contrary, black aspergilli (MIC > 16 μg/mL) showed resistance to ITC in more than 50% of cases, without any clear mechanisms explaining this resistance (30). Our results showed that the common black aspergilli species (e.g., *A. niger* and *A. tubingensis*) showed higher ITC resistance.

Ototopical FLU is used for the management of otomycosis, especially against *Aspergillus* (31). The GM MICs of FLU against *A. niger*, *A. tubingensis*, and *A. flavus* were obtained to be 10.2, 2.47, and 1.14 μg/mL, respectively (Table 1). Our results revealed FLU resistance in 6 isolates (35.29%), which is in line with other reports indicating *in vitro* FLU resistance

Table 1. *In Vitro* Antifungal Susceptibilities of 91 *Aspergillus* Strains Isolated from Otomycosis Against the Eleven Antifungal Agents

Species (No.)	Range	MIC Parameters (μg/mL)			R[a]
		G-mean	MIC_{50}	MIC_{90}	
All *Aspergillus* Strains (n = 91)					-
AMB	0.06 - > 16	4.922	16	32	
NYS	0.25 - 32	13.739	16	32	
FLU	0.12 - 32	3.695	4	16	
ITC	0.25 - > 16	3.568	4	> 16	
VRC	0.06 - > 16	0.377	0.25	2	
KTO	0.25 - > 16	8.311	8	> 16	
CLO	0.06 - 8	0.777	1	2	
EFN	0.031 - 0.5	0.109	0.06	0.25	
LUL	0.031 - 0.5	0.098	0.06	0.25	
CAS	0.25 - > 16	8.503	> 16	> 16	
TRB	0.031 - 16	0.146	0.12	1	
***A. tubingensis* (n = 57)**					-
AMB	0.06 - > 16	3.735	8	> 16	
NYS	0.25 - 32	9.718	8	32	
FLU	0.12 - 8	2.477	4	8	
ITC	0.25 - > 16	3.374	2	> 16	
VRC	0.06 - 2	0.276	0.25	1	
KTO	0.25 - > 16	7.171	8	16	
CLO	0.06 - 2	0.601	1	2	
EFN	0.06 - 0.5	0.127	0.12	0.25	
LUL	0.031 - 0.5	0.120	0.12	0.5	
CAS	0.25 - > 16	4.859	16	> 16	
TRB	0.06 - 8	0.179	0.12	2	
***A. niger* (n = 28)**					
AMB	0.12 - > 16	11.853	16	> 16	27
NYS	8 - 32	23.776	32	32	-
FLU	4 - 32	10.247	8	16	-
ITC	1 - > 16	5.384	8	> 16	18
VRC	0.25 - > 16	0.820	0.5	2	4
KTO	0.5 - > 16	11.888	16	> 16	-
CLO	0.12 - 8	1.806	2	4	-
EFN	0.06 - 0.5	0.099	0.06	0.25	-
LUL	0.06 - 0.25	0.079	0.06	0.25	-
CAS	8 - > 16	26.251	16	> 16	28
TRB	0.031 - 16	0.128	0.06	0.25	-
***A. flavus* (n = 5)**					
AMB	0.25 - 16	0.758	ND	ND	0
NYS	16 - 32	27.858	ND	ND	-
FLU	0.25 - 8	1.149	ND	ND	-
ITC	0.25 - > 16	0.871	ND	ND	1
VRC	0.06 - 0.5	0.186	ND	ND	0
KTO	2 - > 16	6.063	ND	ND	-
CLO	0.06 - 2	0.214	ND	ND	-
EFN	0.06 - 0.031	0.035	ND	ND	-
LUL	0.031	0.031	ND	ND	-
CAS	0.25 - > 16	6.964	ND	ND	4
TRB	0.031 - 0.06	0.040	ND	ND	-
***A. awamori* (n = 1)**					-
AMB	8	ND	ND	ND	
NYS	32	ND	ND	ND	
FLU	4	ND	ND	ND	
ITC	1	ND	ND	ND	
VRC	0.25	ND	ND	ND	
KTO	8	ND	ND	ND	
CLO	0.06	ND	ND	ND	
EFN	0.06	ND	ND	ND	
LUL	0.06	ND	ND	ND	
CAS	> 16	ND	ND	ND	
TRB	0.031	ND	ND	ND	

Abbreviations: MIC_{50}, concentration, at which 50% of the isolates were inhibited; MIC_{90}, concentration, at which 90% of the isolates were inhibited; G-mean, geometric mean; FLU, fluconazole; VRC, voriconazole; ITC, itraconazole; LUL, luliconazole; EFN, efinaconazole; KTO, ketoconazole; NYS, nystatin; TRB, terbinafine; CLO, clotrimazole; AMB, amphotericin B; CAS, caspofungin

[a] R (resistant) based on epidemiological cutoff values for *in vitro* susceptibility testing of various *Aspergillus* spp. using the CLSI M59 method (19).

Table 2. *In Vitro* Antifungal Susceptibilities of 17 *Candida* Strains Isolated from Otomycosis Against the Eleven Antifungal Agents

Species (No.)	Range	MIC Parameters (μg/mL)					
		G-mean	MIC_{50}	MIC_{90}	S	SDD	R
All *Candida* strains (n = 17)							
AMB	0.06 - 8	0.537	0.5	4	-	-	-
NYS	0.06 - 32	9.372	16	32	-	-	-
FLU	0.25 - 64	4.905	4	32	4	7	6
ITC	0.12 - 16	0.655	1	2	-	-	-
VRC	0.031 - > 16	0.475	0.25	4	6	4	1
KTO	0.5 - 8	1.277	1	4	-	-	-
CLO	0.031 - 4	0.219	0.25	0.5	-	-	-
EFN	0.06 - 1	0.144	0.06	0.5	-	-	-
LUL	0.06 - 0.5	0.133	0.06	0.5	-	-	-
CAS	0.031 - 4	0.194	0.12	2	15	-	2
TRB	0.25 - > 16	13.592	>16	>16	-	-	-
***C. parapsilosis* (n = 7)**							
AMB	0.06 - 8	0.403	ND	ND	-	-	-
NYS	0.06 - 32	4.819	ND	ND	-	-	-
FLU	0.25 - 64	2.972	ND	ND	4	-	3
ITC	0.12 - 16	0.244	ND	ND	-	-	-
VRC	0.031 - 32	0.075	ND	ND	5	2	-
KTO	0.5 - 8	2.208	ND	ND	-	-	-
CLO	0.031 - 4	0.136	ND	ND	-	-	-
EFN	0.06 - 1	0.247	ND	ND	-	-	-
LUL	0.06 - 0.5	0.203	ND	ND	-	-	-
CAS	0.031 - 4	0.606	ND	ND	7	-	-
TRB	0.25 - > 16	6.563	ND	ND	-	-	-
***C. glabrata* (n = 6)**							
AMB	0.5 - 8	0.794	ND	ND	-	-	-
NYS	0.06 - 16	8.979	ND	ND	-	-	-
FLU	4 - 64	4.489	ND	ND	-	6	-
ITC	0.12	2	ND	ND	-	-	-
VRC	0.06 - 4	3.564	ND	ND	-	-	-
KTO	0.5 - 1	0.561	ND	ND	-	-	-
CLO	0.031 - 4	0.397	ND	ND	-	-	-
EFN	0.06 - 0.5	0.085	ND	ND	-	-	-
LUL	0.06 - 0.25	0.076	ND	ND	-	-	-
CAS	0.031 - 2	0.069	ND	ND	5	-	1
TRB	> 16	32	ND	ND	-	-	-
***C. albicans* (n = 3)**							
AMB	0.06 - 8	1	ND	ND	-	-	-
NYS	0.06 - 32	32	ND	ND	-	-	-
FLU	0.25 - 64	10.079	ND	ND	-	1	2
ITC	0.12 - 16	1.243	ND	ND	-	-	-
VRC	0.031 - 32	0.783	ND	ND	1	1	1
KTO	0.5 - 8	1.259	ND	ND	-	-	-
CLO	0.031 - 4	0.249	ND	ND	-	-	-
EFN	0.06 - 1	0.097	ND	ND	-	-	-
LUL	0.06 - 0.5	0.122	ND	ND	-	-	-
CAS	0.031 - 4	0.124	ND	ND	2	-	1
TRB	0.25 - > 16	12.699	ND	ND	-	-	-
***C. orthopsilosis* (n = 1)**							
AMB	0.06	0.06	ND	ND	-	-	-
NYS	32	32	ND	ND	-	-	-
FLU	32	32	ND	ND	-	-	1
ITC	0.12	0.12	ND	ND	-	-	-
VRC	0.25	0.25	ND	ND	-	1	-
KTO	4	4	ND	ND	-	-	-
CLO	0.12	0.12	ND	ND	-	-	-
EFN	0.25	0.25	ND	ND	-	-	-
LUL	0.25	0.25	ND	ND	-	-	-
CAS	0.12	0.12	ND	ND	1	-	-
TRB	16	16	ND	ND	-	-	-

Abbreviations: G-mean, geometric mean; FLU, fluconazole; VRC, voriconazole; ITC, itraconazole; LUL, luliconazole; EFN, efinaconazole; KTO, ketoconazole; NYS, nystatin; TRB, terbinafine; CLO, clotrimazole; AMB, amphotericin B; CAS, caspofungin; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

(32). Evidence is suggestive of the higher susceptibility of *A. niger* and *A. tubingensis* accounting for mild to systemic aspergillosis to AMB under *in vitro* conditions. However, these cases undergo frequent relapses and failures in spite of using antifungal therapy; accordingly, their therapeutic values are challenging (33).

In the present study, AMB MICs ranged between 0.06 and > 16 μg/mL for *Aspergillus* species, which is similar to the results reported by Oakley et al., but in contrast to those obtained by Kaya et al. (34, 35). Among the tested antifungal agents, KTO, CAS, and NYS had higher GM MICs against *Aspergillus* species compared to other drugs. Our data showed that *A. flavus* was more susceptible to LUL and EFN than *A. niger* and *A. tubingensis*. Nevertheless, aspergilli recovered from the external auditory canal demonstrated higher susceptibility to LUL, EFN, and TRB. Therefore, these topical agents can be considered as effective treatments for aspergillosis. In this study, the susceptibility of *Candida* isolates recovered from otomycosis was tested for the eleven drugs. The LUL (GM = 0.133μg/mL) and EFN (GM = 0.144 μg/mL) had the lowest MICs for *Candida* isolates. The LUL and EFN have an unclear sensitivity, dose dependence, or resistance range because of the lack of an acceptable breakpoint for *Candida* species.

The GM MICs of LUL and EFN for *C. glabrata* were obtained to be 0.076 and 0.085 μg/mL, respectively. These values were 0.122 and 0.097 μg/mL for *C. albicans* and 0.203 and 0.247 μg/mL for *C. parapsilosis*, respectively. Our results revealed that *C. glabrata* had lower MICs for LUL and EFN than *C. parapsilosis* and *C. albicans* strains. Niwano et al. (36) and Koga et al. (37) were the first researchers reporting a very low MIC for LUL and EFN (MIC range: 0.031 - 0.13 and 0.031 - 0.25 μg/mL, respectively) against *C. albicans*. Niwano et al., comparing oral LUL and FLU in a murine model of systemic candidiasis, reported the lower efficacy of LUL compared to that of FLU (36, 38). In the current study, CAS had a GM MIC of 0.194 μg/mL against *Candida* species. The previous studies have also confirmed the potent *in vitro* activity of this drug against *Candida* strains (39). Based on our results, NYS and TRB had the highest MIC values against *Candida* isolates, which is consistent with the results reported in the literature (40). However, *Candida* isolates also showed resistance to FLU, VRC, and CAS.

5.1. Conclusions

Our findings revealed that LUL and EFN had the highest *in vitro* activities against *Aspergillus* (GM MICs of 0.098 and 0.109 μg/mL, respectively) and *Candida* (GM MICs of 0.133 and 0.144 μg/mL, respectively) strains. In most of the cases, the novel topical triazoles and imidazoles (EFN and LUL) appeared to show good antifungal activities. The findings of the present research could be useful in promoting the significance of antifungal susceptibility testing for appropriate treatment.

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Footnotes

Authors' Contribution: S.A and Gh.Sh designed and drafted the manuscript, Gh.Sh, R.R, M.E, B.A, S.G performed the experiments, Gh.Sh, S.A, and J.J analyzed the data, Gh.Sh, S.N-S, A.R-M, S.A, J.J, B.A, S.N.T, M.E, F.H. and S.N.T interpreted the results, and Gh.Sh and S.A wrote the original draft of the manuscript. All authors have read and approved the final version of the manuscript.

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