The potency of luliconazole against clinical and environmental Aspergillus nigri complex

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ABSTRACT

Background and Objectives: Black Aspergillus strains including, Aspergillus niger and A. tubingensis, are the most cause of otomycosis with worldwide distribution. Although, amphotericin B was a Gold standard for the treatment of invasive fungal infection for several decades, it gradually replaced by fluconazole and /or voriconazole. Moreover, luliconazole, appears to offer the best potential for in vitro activity against black Aspergillus strains. The aim of the present study was to compare the in vitro activity luliconazole, with commonly used antifungals against clinical and environmental strains of black Aspergillus.

Materials and Methods: Sixty seven (37 clinical and 30 environmental) strains of black Aspergillus were identified using morphological and molecular technique (β-Tubulin gene). In addition, antifungal susceptibility test was applied according to CLSI M38 A2. The results were reported as minimum inhibitory concentration (MIC) or minimum effective concentration (MEC) range, MIC50 or MEC50, MIC90 or MEC90 and MIC geometric (GM) or MECGM.

Results: Aspergillus niger was the common isolate followed by, A. tubingensis in both clinical and environmental strains. The lowest MIC range, MIC50, MIC90, and MICGM was attributed to luliconazole in clinical strains. The highest resistant rate was found in amphotericin B for both clinical (86.5%) and environmental (96.7%) strains whereas 54.1% of clinical and 30% of environmental isolates were resistant to caspofungin. Clinical strains of Aspergillus were more sensitive to voriconazole (86.7%) than environmental strains (70.3%). On the other hand, 83.8% of clinical and 70% of environmental isolates were resistant to posaconazole.

Conclusion: Luliconazole versus amphotericin B, voriconazole, posaconazole and caspofungin is a potent antifungal for Aspergillus Nigri complex. The in vitro extremely antifungal efficacy against black Aspergillus strains of luliconazole, is different from those of other used antifungals.

Keywords: Black Aspergillus strains; Luliconazole; Clinical and environmental isolates; Antifungal profile
INTRODUCTION

Luliconazole (Luzu®, (-)-(E)-[(4R)-4-(2,4-dichlorophe-nyl)-1,3-dithiolan-2-ylidene] (1H-imidaz-ol-1-yl) acetonitrile), is an imidazole antifungal with molecular formula: C_{14}H_{9}Cl_{2}N_{3}S_{2} (1). Luliconazole was basically introduced as anti-dermatophytic antifungal in Japan and India (1, 2). However, it has demonstrated activity in vitro against multiple Aspergillus species, including Aspergillus fumigatus (3, 4), A. terreus (4, 5), A. flavus (4, 6), A. niger (4) and A. tubingensis (4). The availability of a novel antifungal, luliconazole, appears to offer the potential for improved therapy for a wide range of invasive fungal infections, including aspergillosis, dermatophytosis, and onychomycosis (2, 7, 8).

While, amphotericin B was a Gold standard in the first-line treatment of invasive fungal infections for several decades (9), it has been replaced by several new antifungals including, voriconazole, posaconazole and caspofungin (10, 11). Voriconazole was presented as the primary therapy for invasive pulmonary aspergillosis in a clinical trials (12). Further studies have shown that posaconazole is a useful antifungal for invasive fungal infection including aspergillosis (13). On the other hand, during 2-3 last decades, caspofungin was developed to improve the potency of each antifungal against clinical and environmental strains of black Aspergillus. Furthermore, the potency of each antifungal against clinical and environmental isolates was compared.

MATERIALS AND METHODS

Fungal isolates. Thirty seven clinical isolates of black Aspergillus strains were previously isolated from otomycosis samples, identified based on morphology characteristics and preserved at Medical Mycology laboratory affiliated to Ahvaz Jundishapur University of Medical Sciences. This project was approved by the ethical committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1396.1066).

Environmental strains of black Aspergillus (30 strains) were trapped from airborne spores using Sabouraud dextrose agar (SDA) (BioLife, Italia) plates. Primary screening of black Aspergillus strains was applied based on macroscopic (Black colony) and microscopic morphology. All strains (clinical and environmental) were subcultured on SDA and re-identified using molecular tests.

DNA extraction. All strains (clinical and environment isolates) were subcultured on SDA plates and incubated at 29°C for 24-48 hours. Mycelia were collected in cryo-tubes containing 300 µL lysis buffer and 0.46 g glass beads and kept at 4°C for 72 hours. The tube contents were homogenized using a Speed-Mill PLUS Homogenizer (Analytikjena, Germany) for 6 minutes (3 cycles) and boiled at 100°C for 20 minutes. 300 µL of sodium acetate (3M) was added to each tube and stored at -20°C for 10 minutes. Supernatants were removed after a centrifugation at 12000 rpm for 10 minutes. DNA was purified using phenol-chloroform-isooamyl alcohol (Merck, Germany) according to a protocol devised by Makimura et al. (30). Finally purified DNA was preserved at -20°C for further tests.

Molecular identification. β-Tubulin gene was
used for the molecular detection of strains using primers pair, \( \beta_{\text{t}2\text{a}} \) (forward), 5' GGTAACCAAATC-GGTGCTGCTTTC 3' and \( \beta_{\text{t}2\text{b}} \) (reverse) 5' ACCCT-CAGTGTAGTACCCCTTGGC 3' (31). PCR products subjected for sequence analysis and then sequences were manually verified by MEGA6 software package (https://www.megasoftware.net/) and aligned using the CLUSTALW algorithm. All sequences were compared to reference sequences in the GenBank (NCBI) and CBS database via the nucleotide BLAST™ algorithm to obtain a definitive identification (similarity values ≥ 99%). Finally, all nucleotide sequences representative were deposited in the GenBank database.

**Antifungal susceptibility assay.** Twofold serial dilutions of antifungals including, luliconazole (APIChem Technology, China) (from 0.00012 to 0.25 \( \mu \text{g/mL} \)), amphotericin B (Sigma - Aldrich, Germany) (from 0.125 to 16 \( \mu \text{g/mL} \)), voriconazole (Sigma - Aldrich, Germany) (from 0.0078 to 4 \( \mu \text{g/mL} \)), posaconazole (Sigma - Aldrich, Germany) (from 0.0312 to 4 \( \mu \text{g/mL} \)), and caspofungin (Sigma - Aldrich, Germany) (from 0.0078 to 1 \( \mu \text{g/mL} \)) were prepared in RPMI 1640 (Bio Idea, Iran). Antifungal susceptibility test was performed according to CLSI M38 A2 (32). A standard suspension (0.5 McFarland) of 48-72 hours cultures on SDA was prepared in sterile saline (0.85%) with 0.2% Tween 20 (Merck, Germany). Then, 100 \( \mu \text{L} \) of diluted suspension (1:50) and 100 \( \mu \text{L} \) of serial dilutions of each antifungal were added to each well of 96-well microplates. Microplates incubated at 35ºC for 24-72 hours and results were recorded as MIC or minimum effective concentration (MEC). Finally, MIC or MEC range, \( \text{MIC}_{50} \), \( \text{MIC}_{90} \), \( \text{MIC}_{\text{GM}} \) and \( \text{MEC}_{\text{GM}} \) were calculated. CLSI or EUCAST have not been defined any clinical or epidemiologic breakpoints/cut-offs for amphotericin B, voriconazole, posaconazole, caspofungin and *Aspergillus* species. Strains susceptibility or resistance to each antifungals was evaluated according to commonly utilized breakpoints (Table 1) (33-38).

**Statistical analysis.** The Chi-squared test using the Social Science Statistics software (Online) was applied to determine the significant between variables and \( P \) value < 0.05 is considered as significance level.

### Table 1. Defined breakpoints of amphotericin B, voriconazole, posaconazole and caspofungin for *Aspergillus niger* sensu lato

| Antifungals     | MIC or MEC (\( \mu \text{g/mL} \)) | Sensitive | Resistance |
|-----------------|------------------------------------|-----------|------------|
| Amphotericin B  | ≤2                                 | >2        |
| Posaconazole    | ≤0.5                               | >0.5      |
| Voriconazole    | ≤1                                 | >1        |
| Caspofungin     | ≤0.06                              | >0.06     |
| Luliconazole    | Undefined                          | Undefined |

**RESULTS**

**Molecular detection of isolates.** 37 clinical isolates of black *Aspergillus* were detected using molecular and sequencing techniques. *Aspergillus niger* (21, 56.8%) was the common strain followed by, *A. tubingensis* (11, 29.8%), *A. luchuensis* (1, 2.7%), and black *Aspergillus* strains (4, 10.8%) (Table 2). Furthermore, out of 30 environmental black *Aspergillus* isolates, 15 (50%) was identified as *A. niger* followed by, *A. tubingensis* (13, 43.3%), *A. piperis* (1, 3.3%) and black *Aspergillus* strains (1, 3.3%). However, we could not identified four clinical and one environmental black *Aspergillus* strains, using molecular technique due to inadequate DNA sample size.

**Clinical isolates.** The lowest MIC range (0.00024-0.125 \( \mu \text{g/mL} \)), \( \text{MIC}_{50} \) (0.00195 \( \mu \text{g/mL} \)), \( \text{MIC}_{90} \) (0.125 \( \mu \text{g/mL} \)) and \( \text{MIC}_{\text{GM}} \) (0.00295 \( \mu \text{g/mL} \)) was attributed to luliconazole (Table 3). The MEC range for all clinical *Aspergillus* species was 0.0078-1 \( \mu \text{g/mL} \) for caspofungin. In addition, the 50% and 90% MEC (\( \text{MEC}_{50} \), \( \text{MEC}_{90} \)) values were 0.125 and 0.5 \( \mu \text{g/mL} \) for caspofungin, respectively. Totally, the 54.1% of isolates were resistant to caspofungin. The results have shown that the MIC range of amphotericin B for tested isolates was 0.25-16 \( \mu \text{g/mL} \). However, \( \text{MIC}_{90} \) was similar, 8 \( \mu \text{g/mL} \). The highest resistant rate (86.5%) was found for amphotericin B. The MIC ranges for clinical isolates of black *Aspergillus* strains were 0.0078-4 and 0.0625-4 \( \mu \text{g/mL} \) of voriconazole and posaconazole, respectively. However, the \( \text{MIC}_{\text{GM}} \) for voriconazole (0.77 \( \mu \text{g/mL} \)) was lower than posaconazole (1.45 \( \mu \text{g/mL} \)). In our
Table 2. Clinical and environmental black *Aspergillus* strains

| Sources                        | Morphological identification | Molecular identification                      |
|--------------------------------|------------------------------|-----------------------------------------------|
| Clinical isolates (37 isolates)| *Aspergillus niger sensu lato* | *A. niger*, sensu stricto (21)                 |
|                               |                              | *A. tubingensis* (11)                          |
|                               |                              | *A. luchuensis* (1)                            |
|                               |                              | Black *Aspergillus* strains (4)                |
| Environmental isolates (30 isolates) | *Aspergillus niger sensu lato* | *A. niger*, sensu stricto (15)                 |
|                               |                              | *A. tubingensis* (13)                          |
|                               |                              | *A. piperis* (1)                               |
|                               |                              | Black *Aspergillus* strains (1)                |

Table 3. The antifungal susceptibility pattern of 67 (37 clinical and 30 environmental) strains of black *Aspergillus*

| Clinical isolates of *Aspergillus* (37 isolates) | Luliconazole | N | MIC range (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MICGM (µg/mL) | R (%) |
|-------------------------------------------------|--------------|---|-------------------|---------------|---------------|---------------|---------------|-------|
| A. niger                                         | 21           | 0.000024 - 0.125 | 0.000195 | 0.125 | 0.00378 | - |
| A. tubingensis                                   | 11           | 0.000024 - 0.125 | 0.000195 | 0.00391 | 0.00251 | - |
| A. luchuensis                                    | 1            | 0.00098 | - | - | - | - |
| Black *Aspergillus*                              | 4            | 0.00049 - 0.00391 | - | - | - | - |
| Total                                           | 37           | 0.000024 - 0.125 | 0.000195 | 0.125 | 0.00295 | - |

| Ampicillin B | N | MIC range (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MICGM (µg/mL) | R (%) |
|--------------|---|-------------------|---------------|---------------|---------------|---------------|-------|
| A. niger     | 21 | 0.25 - 8 | 8 | 8 | 4.56 | 17 (81%) |
| A. tubingensis | 11 | 4 - 16 | 8 | 8 | 11 (100%) |
| A. luchuensis | 1 | 1 | - | - | - | - |
| Black *Aspergillus* | 4 | 4 - 8 | - | - | - | - |
| Total        | 37 | 0.25 - 16 | 8 | 8 | 5 | 32 (86.5%) |

| Voriconazole | N | MIC range (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MICGM (µg/mL) | R (%) |
|--------------|---|-------------------|---------------|---------------|---------------|---------------|-------|
| A. niger     | 21 | 0.0625 - 2 | 1 | 2 | 0.99 | 5 (23.8%) |
| A. tubingensis | 11 | 0.5 - 4 | 1 | 2 | 1.20 | 4 (36.4%) |
| A. luchuensis | 1 | 0.0078 | - | - | - | - |
| Black *Aspergillus* | 4 | 0.5 - 2 | - | - | - | - |
| Total        | 37 | 0.0078 - 4 | 1 | 2 | 0.77 | 11 (29.7%) |

| Posaconazole | N | MIC range (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MICGM (µg/mL) | R (%) |
|--------------|---|-------------------|---------------|---------------|---------------|---------------|-------|
| A. niger     | 21 | 0.0625 - 4 | 2 | 2 | 1.26 | 17 (81%) |
| A. tubingensis | 11 | 0.125 - 4 | 2 | 4 | 2.13 | 10 (90.9%) |
| A. luchuensis | 1 | 0.5 | - | - | - | - |
| Black *Aspergillus* | 4 | 0.25 - 4 | - | - | - | - |
| Total        | 37 | 0.0625 - 4 | 2 | 4 | 1.45 | 31 (83.8%) |

| Caspofungin | N | MIC range (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MICGM (µg/mL) | R (%) |
|-------------|---|-------------------|---------------|---------------|---------------|---------------|-------|
| A. niger    | 21 | 0.0078 - 1 | 0.125 | 0.5 | 0.099 | 11 (52.4%) |
| A. tubingensis | 11 | 0.032 - 0.5 | 0.125 | 0.5 | 0.133 | 7 (63.6%) |
| A. luchuensis | 1 | 0.032 | - | - | - | - |
| Black *Aspergillus* | 4 | 0.0625 - 0.25 | - | - | - | - |
| Total       | 37 | 0.0078 - 1 | 0.125 | 0.5 | 0.107 | 20 (54.1%) |

Environmental isolates of *Aspergillus* (30 isolates)

| Luliconazole | N | MIC range (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MIC₉₀ (µg/mL) | MICGM (µg/mL) | R (%) |
|--------------|---|-------------------|---------------|---------------|---------------|---------------|-------|
| *Aspergillus niger* | 15 | 0.00098 - 0.0078 | 0.00195 | 0.00391 | 0.00214 | - |
| *A. tubingensis* | 13 | 0.00049 - 0.00781 | 0.00195 | 0.00391 | 0.00195 | - |
Table 3. Continuing...

|                  | N   | MIC range (µg/mL) | MIC$_{50}$ (µg/mL) | MIC$_{90}$ (µg/mL) | MICGM (µg/mL) | R (%)     |
|------------------|-----|------------------|--------------------|--------------------|---------------|-----------|
| **Amphotericin B** |     |                  |                    |                    |               |           |
| A. niger         | 15  | 0.125 - 2        | 1                  | 2                  | 0.6300        | 2 (13.3%) |
| A. tubingensis   | 13  | 0.0625 - 2       | 0.5                | 2                  | 0.4261        | 2 (15.4%) |
| A. piperis       | 1   | 0.125            | -                  | -                  | -             | -         |
| Black Aspergillus| 1   | 0.0625           | -                  | -                  | -             | -         |
| Total            | 30  | 0.0625 - 2       | 0.5                | 2                  | 0.4665        | 4 (13.3%) |
| **Voriconazole** |     |                  |                    |                    |               |           |
| A. niger         | 15  | 0.5 - 4          | 2                  | 4                  | 1.8234        | 14 (93%)  |
| A. tubingensis   | 13  | 0.125 - 4        | 2                  | 4                  | 1.1125        | 7 (53.8%) |
| A. piperis       | 1   | 0.5              | -                  | -                  | -             | -         |
| Black Aspergillus| 1   | 0.0625           | -                  | -                  | -             | -         |
| Total            | 30  | 0.0625 - 4       | 2                  | 4                  | 1.2599        | 21 (70%)  |
| **Posaconazole** |     |                  |                    |                    |               |           |
| A. niger         | 15  | 0.0078 - 0.25    | 0.032              | 0.25               | 0.0412        | 3 (20%)   |
| A. tubingensis   | 13  | 0.0078 - 0.5     | 0.0625             | 0.5                | 0.0733        | 6 (46.2%) |
| A. piperis       | 1   | 0.0625           | -                  | -                  | -             | -         |
| Black Aspergillus| 1   | 0.0078           | -                  | -                  | -             | -         |
| Total            | 30  | 0.0078 - 0.5     | 0.0625             | 0.25               | 0.0507        | 9 (30%)   |

N, number; MEC, Minimum effective concentration; MIC, Minimum inhibitory concentration; GM, Geometric; R, Resistant

study, 29.7% and 83.8% of isolates were resistant to voriconazole and posaconazole, respectively.

**Environmental isolates.** The results summarized in Table 3 show the in vitro susceptibilities of 30 environmental Aspergillus Nigrii against several antifungals. The same as clinical isolates, the lowest MIC range was 0.00049-0.00781 µg/ml for luliconazole. Moreover, the MIC$_{50}$, MIC$_{90}$ and MICGM of luliconazole were 0.00195, 0.00391 and 0.00195 µg/ml, respectively. The MEC range, MEC$_{50}$, MEC$_{90}$ and MECGM for caspofungin were 0.0078-0.5, 0.0625, 0.25, and 0.0507 µg/ml, respectively. Furthermore, 30% of environmental strains were resistant to caspofungin. As shown in Table 3, the MIC range for amphotericin B was 2-16 µg/ml followed by, MIC$_{50}$, MIC$_{90}$ and MICGM were 8, 8 and 6.063 µg/ml, respectively. Moreover, 96.7% of strains were resistant to amphotericin B. Totally, the MIC range voriconazole for environmental isolates of *Aspergillus* was 0.0625-2 µg/ml, whereas MIC$_{50}$ 2 µg/ml, MIC$_{90}$ 0.5 and MICGM 0.4665 µg/ml. Our results indicated that only 4 (13.3%) strains were resistant to voriconazole. The tested isolates were inhibited at MIC range 0.0625-4 µg/ml by posaconazole. Furthermore, the MIC$_{50}$, MIC$_{90}$ and MECGM were 2, 4 and 1.2599 µg/ml, respectively. In addition, 70% of strains were resistant to posaconazole.

Caspofungin was significantly more effective against environmental than clinical strains (P = 0.048) of black *Aspergillus* strains. However, the inhibitory effect of amphotericin B, posaconazole and voriconazole was similar against both tested strains (clinical and environmental) (amphotericin
B, $P=0.147$; voriconazole, $P=0.109$; posaconazole, $P=0.178$). When we compared the effect antifungals against \textit{A. niger} and \textit{A. tubingensis} strains, it found that caspofungin was more effective on \textit{A. niger} with environmental sources than clinical strains ($P=0.0482$). Whereas, the effect of other antifungals against both species was not significant.

Our results showed that 32 (86.5\%) of clinical strains were resistant to 2, 3 or 4 antifungals, 2 (5.4\%) isolates were resistant to one antifungal and 3 (8.1\%) isolates were fully susceptible to all antifungals (Table 4). Two strains of \textit{A. tubingensis}, one \textit{A. niger} and one black \textit{Aspergillus} strains were resistant to all antifungals (except luliconazole). On the other hand, 21 (70\%) of environmental strains were resistance to 2 - 4 antifungals and only 30% of strains were resistance to one antifungal (Table 5). Two strains of \textit{A. niger} and one \textit{A. tubingensis} were resistant to all antifungals (except luliconazole).

**DISCUSSION**

\textit{Aspergillus} strains isolated from clinical and air borne samples were identified using classical morphological features and molecular methods. In the present study, \textit{A. tubingensis}, \textit{A. luchuensis} and \textit{A. piperis} were identified as the cryptic species of \textit{A. niger} sensu lato by the sequence analysis of $\beta$-tubulin gene. Several reports have shown that \textit{A. niger} is generally as common causative agent of otomycosis and one of the most important agent for invasive aspergillosis (20, 22, 26, 39-41). However, this species cannot be reliably detected from other cryptic members of \textit{Aspergillus} section Nigri using conventional morphological methods. Molecular tools with sequence-based techniques such as partial sequence of the $\beta$-tubulin gene are presented as the most valuable method for \textit{A. niger} Nigri species assignment (4, 21). These molecular techniques are indicating that this species comprises 19 cryptic species (4, 16, 21) with more prevalence of \textit{A. niger} sensu stricto and \textit{A. tubingensis} (16, 42).

Our results showed that, although the luliconazole MIC ranges for strains were extremely low, this range for environmental strains (0.00781-0.00049 $\mu$g/ml) was lower than clinical strains (0.125 - 0.00024 $\mu$g/ml). As shown in Table 5, only five clinical strains (\textit{A. niger} sensu stricto, 4 isolates and \textit{A. tubingensis}, 1 isolate) have a MIC = 0.125 $\mu$g/ml. 30/30 (100\%) of environmental and 83.8\% of clinical strains had the lowest MICs (MICs < 0.00781 $\mu$g/ml) against luliconazole. Moreover, the MICGM for environmental

**Table 4. Drug resistance against tested antifungals among 37 clinical strains**

| Clinical strains | Accessions numbers | LUL | POS | VOR | AMP | CAS |
|------------------|--------------------|-----|-----|-----|-----|-----|
| \textit{Aspergillus niger} LC441157 | 0.125 | R | S | R | R |
| \textit{A. niger} LC456335 | 0.125 | S | S | R | R |
| \textit{A. niger} LC456339 | 0.125 | S | S | R | R |
| \textit{A. niger} LC441167 | 0.125 | R | S | R | R |
| \textit{A. tubingensis} LC456340 | 0.125 | R | S | R | R |
| \textit{A. niger} LC456341 | 0.0156 | R | S | R | R |
| \textit{A. niger} LC441156 | 0.0078 | R | S | R | R |
| \textit{A. niger} LC456337 | 0.0078 | R | S | S | S |
| \textit{A. tubingensis} LC456338 | 0.0039 | R | R | R |
| Black \textit{Aspergillus} LC441158 | 0.0039 | R | S | S | S |
| \textit{A. tubingensis} LC441168 | 0.0039 | R | R | R | R |
| \textit{A. niger} LC441162 | 0.0039 | R | S | R | R |
| \textit{A. niger} LC456326 | 0.0019 | R | R | S | S |
| \textit{A. tubingensis} LC456298 | 0.0019 | R | R | S | S |
| \textit{A. tubingensis} LC456302 | 0.0019 | R | R | S | S |
| Black \textit{Aspergillus} LC441157 | 0.0019 | R | R | R | R |
| \textit{A. tubingensis} LC456301 | 0.0019 | R | S | S | S |
| \textit{A. niger} LC441161 | 0.0019 | R | S | S | S |
| \textit{A. tubingensis} LC441169 | 0.0019 | R | S | S | S |
| \textit{A. niger} LC441158 | 0.0019 | R | R | R | R |
| \textit{A. tubingensis} LC456303 | 0.0019 | R | S | S | S |
| \textit{A. niger} LC456323 | 0.0019 | R | R | R | R |
| Black \textit{Aspergillus} LC456336 | 0.0019 | R | R | R | R |
| \textit{A. tubingensis} LC456171 | 0.0019 | R | S | S | S |
| \textit{A. niger} LC441163 | 0.0009 | R | S | S | S |
| \textit{A. niger} LC441159 | 0.0009 | R | S | S | S |
| \textit{A. niger} LC441160 | 0.0009 | R | S | S | S |
| \textit{A. niger} LC441165 | 0.0009 | R | R | R | R |
| \textit{A. tubingensis} LC441170 | 0.0009 | R | S | S | S |
| \textit{A. niger} LC441164 | 0.0009 | R | S | S | S |
| \textit{A. niger} LC456320 | 0.0009 | R | S | S | S |
| \textit{A. luchuensis} LC456304 | 0.0009 | S | S | S | S |
| Black \textit{Aspergillus} LC456326 | 0.0009 | S | R | R | R |
| \textit{A. tubingensis} LC456297 | 0.0024 | R | S | R | R |
| \textit{A. niger} LC441166 | 0.0024 | S | S | S | S |
| \textit{A. niger} LC441155 | 0.0024 | S | S | S | S |

LUL, Luliconazole; POS, Posaconazole; VOR, Voriconazole; AMP, Amphotericin B; CAS, Caspofungin; R, Resistance; S, Susceptible
In conclusion, luliconazole versus amphotericin B showed a significant difference in the resistance pattern of environmental and clinical isolates. The efficacy of amphotericin B and voriconazole in Misra et al. (30) was comparable to our results. Araujo et al. (21) found the lowest MICs of luliconazole against black Aspergillus strains at 0.008 - 0.063 μg/ml. In agreement with our study, Hashimoto et al. (15) revealed no remarkable differences between the MIC distribution rate of voriconazole against clinical and environmental isolates. Furthermore, all tested A. niger (environment and clinical isolates) were susceptible to both amphotericin B and voriconazole in Misra et al., research (47). Aspergillus tubingensis resistant strains to amphotericin B were very common both in environmental and clinical settings, followed by posaconazole, caspofungin, and voriconazole. However, the resistant rate to amphotericin B was lower among environmental than clinical strains. Hashimoto et al. finding suggests that A. tubingensis is intrinsically resistant to azole antifungals (15). Antifungal susceptibility testing of our A. tubingensis strains revealed 90.9% and 53.8% of clinical and environmental isolates were resistant to posaconazole.

CONCLUSION

In conclusion, luliconazole versus amphotericin

and clinical strains were 0.00195 and 0.00295 μg/ml, respectively. Some studies have shown a high efficacy of luliconazole against dermatophytes and onychomycosis agents both in vivo and in vitro (1, 2, 7, 8, 43). Furthermore, recently a few studies examined the potency of luliconazole against different species of Candida, A. fumigatus, A. terreus and Fusarium species (5, 6, 44, 45). However, the potency profile of luliconazole against A. niger complex is unknown. Abastabar et al. (3) and Omran et al. (6) were tested luliconazole against A. fumigatus and A. flavus, and found that the antifungal has the lowest MICs against A. fumigatus (MIC90 0.002 μg/ml) and A. flavus (MIC90 0.032 μg/ml), respectively.

There are the limited data in in vitro efficacy of caspofungin against black Aspergillus strains from clinical and environmental sources. While, the clinical and environmental strains had the same MIC ranges for caspofungin, the resistant to antifungal showed the clear differences between clinical and environmental strains (P = 0.048), where the clinical isolates showed higher resistant rate than the environmental strains. In a report by Badali et al. only 6.1% of environmental strains of A. niger were resistant to caspofungin and all clinical isolates ranged at 0.008 - 0.063 μg/ml (21). In agree with our study Araujo et al., revealed significantly higher MIC values to caspofungin in the case of non-fumigatus clinical than environmental strains (46).

The in vitro activities of posaconazole, voriconazole, and amphotericin B against clinical Aspergillus strains have been reported by Arikan et al. (10). They reported that voriconazole was the most active antifungal against A. niger. Comparable to our results, voriconazole was more potent than the other tested antifungals (with exception luliconazole) against both clinical and environmental strains. Similar to our study, Hashimoto et al., showed no remarkable differences between the MIC distribution rate of voriconazole against clinical and environmental isolates (15). Furthermore, all tested A. niger (environment and clinical isolates) were susceptible to both amphotericin B and voriconazole in Misra et al., research (47). Aspergillus tubingensis resistant strains to amphotericin B was very common both in environmental and clinical settings, followed by posaconazole, caspofungin, and voriconazole. However, the resistant rate to amphotericin B was lower among environmental than clinical strains. Hashimoto et al. finding suggests that A. tubingensis is intrinsically resistant to azole antifungals (15). Antifungal susceptibility testing of our A. tubingensis strains revealed 90.9% and 53.8% of clinical and environmental isolates were resistant to posaconazole.

CONCLUSION

In conclusion, luliconazole versus amphotericin

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B, voriconazole, posaconazole and caspofungin is a potent antifungal for *Aspergillus* Nigri complex. The *in vitro* extremely antifungal efficacy against black *Aspergillus* strains of luliconazole, is different from those of other used antifungals. The MIC range, MIC$_{50}$, MIC$_{90}$ and MICGM of luliconazole against black *Aspergillus* strains were the lowest among the representative tested antifungals. These results suggest luliconazole can be a viable option for the treatment of infections due to black *Aspergillus* strains and should be further investigated *in vivo*.

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