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High prevalence of clinical and environmental triazole-resistant Aspergillus fumigatus in Iran: Is it a challenging issue?

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Abstract

Triazole antifungal agents are the mainstay of aspergillosis treatment. As highlighted in numerous studies, the global increase in the prevalence of triazole resistance could hamper the management of aspergillosis. In the present three-year study, 513 samples (213 clinical and 300 environmental samples) from ten provinces of Iran were processed and screened in terms of azole resistance (4 and 1 mg/L of itraconazole and voriconazole, respectively), using selective plates. Overall, 150 A. fumigatus isolates (71 clinical and 79 environmental isolates) were detected. The isolates were confirmed by partial sequencing of β-tubulin gene. Afterwards, *in vitro* antifungal susceptibility tests against triazole agents were performed based on the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document. The CYP51A gene was sequenced in order to detect mutations. The minimum inhibitory concentration of itraconazole against 10 (6.6%) strains, including clinical (n=3, 4.2%) and environmental (n=7, 8.8%) strains, was higher than the breakpoint and epidemiological cut-off value. Based on the findings, the prevalence of azole-resistant *A. fumigatus* in Iran has remarkably increased from 3.3% to 6.6% in comparison with earlier epidemiological research. Among resistant isolates, TR34/L98H mutations in the cyp51A gene were the most prevalent (n=8, 80%), whereas other point mutations (F46Y, G54W, Y121F, G138C, M172V, F219C, M220I, D255E, T289F, G432C, and G448S mutations) were not detected. Although the number of patients affected by azole-resistant *A. fumigatus* isolates was limited, strict supervision of clinical azole-resistant *A. fumigatus* isolates and persistent environmental screening of azole resistance are vital to the development of approaches for the management of azole resistance in human pathogenic fungi.

**Keywords:** A. fumigatus, Triazole-resistant, TR34/L98H mutation, Iran

**Running title:** High Prevalence of Azole-resistant *A. fumigatus* in Iran
Introduction

Recently, emergence of triazole-resistant *Aspergillus fumigatus* infections has become a major medical concern due to the long term use of azole antifungals. Frequent exposure of *A. fumigatus* to fungicides can lead to the development of azole-resistant species in the environment over time (Verweij et al., 2009b). The first case of azole-resistant *A. fumigatus* was reported in the United Kingdom (Denning et al., 1997). The frequency of azole resistance has increased over the past decade, resulting in the emergence of triazole-resistant *A. fumigatus* infections in European countries (e.g., Austria, Belgium, Denmark, France, Germany, the Netherlands, Norway, Spain, and Turkey), the United States, South America, and Asian countries (e.g., China, India, Iran, Japan, and Kuwait), with an incidence rate of 3.3-38% (Mortensen et al., 2010, Burgel et al., 2012, Badali et al., 2013, Bader et al., 2013, Chowdhary et al., 2011, Seyedmousavi et al., 2013, Lockhart et al., 2011, Chrysanthou, 1997, Pham et al., 2014, Mellado et al., Snelders et al., 2008) (Bueid et al., 2010, Howard et al., 2009, Van der Linden et al., 2011, van Ingen et al., 2014). According to a recent international surveillance study on the clinical isolates of azole-resistant *A. fumigatus*, an incidence rate of 3.2% was reported, which introduced a novel phase in the management of invasive aspergillosis (van der Linden et al., 2015). In addition, according to a nationwide surveillance study, prevalence of azole resistance was estimated at 5.5% in patients with aspergillosis in Belgium (Vermeulen et al., 2015). In a previous study in the Netherlands, prevalence of azole resistance ranged between 5% and 10% in different hospitals (Lestrade et al., 2016). Also, in Denmark, 4.5% of *A. fumigatus* isolates from patients showed triazole resistance, while in France; resistant isolates were detected in 8% of patients with cystic fibrosis. Additionally, in a worldwide survey of medical centers, a resistance rate of 5.8% was reported, with most resistant isolates reported from China (Bowyer and Denning, 2014).
One of the major mechanisms involved in the azole resistance of *A. fumigatus* is the modification of sterol 14α-demethylation, which is a key enzyme in the ergosterol biosynthetic pathway, encoded by *CYP51A* and *CYP51B* genes (Chen et al., 2005, Gulshan and Moye-Rowley, 2007). In fact, mutations at codons TR34/L98H, G54, M220, G138C, and G432C have been identified in azole-resistant *A. fumigatus* strains (Chen et al., 2005, Gulshan and Moye-Rowley, 2007). Over 90% of azole-resistant *A. fumigatus* isolates harbor a leucine-to-histidine substitution at codon 98, along with a 34-bp tandem repeat in the *CYP51A* promoter region. This issue, which is associated with high rates of treatment failure, has become a major medical concern (Van der Linden et al., 2011, Howard et al., 2009, Snelders et al., 2009, Verweij et al., 2007). However, a novel *CYP51A*-promoter duplication mutation, known as TR46/Y121F/T289A, has been recently introduced, which is considered responsible for the elevated minimum inhibitory concentration (MIC) of voriconazole (van der Linden et al., 2013, Chowdhary et al., 2013). Considering the rapid expansion of triazole resistance in various regions and the undesirable outcomes of patient management, we aimed to study the epidemiology of triazole-resistant *A. fumigatus* and *CYP51A* mutations in viable clinical and environmental isolates in Iran during 2013-2015.

**Materials and Methods**

**Fungal strains**

In the present study, 213 clinical specimens were obtained from the lower respiratory tract (*n*=144; 67.6%), sinus (*n*=24; 11.2%), cerumen (*n*=24; 11.2%), nails (*n*=12; 5.6%), and biopsy samples (*n*=9; 4.2%) via routine diagnostic procedures at hospital laboratories in ten provinces of Iran. In addition, 300 environmental samples from the soil of gardens surrounding the hospitals (*n*=190; 63.3%) and indoor air of hospital wards (*n*=110; 36.6%) were collected and examined in terms of the growth of triazole-resistant *A. fumigatus* isolates.
Cultures were prepared on a Sabouraud dextrose agar plate (SDA, Difco, Franklin Lakes, NJ, USA), supplemented with 4 and 1 mg/L of itraconazole and voriconazole, respectively at 45°C for 72 h in the dark. All grown colonies on the plates, mimicking A. fumigatus complexes, were sub-cultured. Strain identities were reconfirmed by DNA sequencing of the partial b-tubulin (BTU) gene using TUB2a [5′-TGACCCAGCAGATGTT-3′] and TUB2b [5′-GTTGTTGGAATCCACTC-3′] as already described (Badali et al., 2013).

**In vitro antifungal susceptibility tests**

The minimum effective concentration (MEC) of caspofungin (Merck Sharp & Dohme, Haarlem, the Netherlands) and MICs of itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer Central Research, Sandwich, United Kingdom), posaconazole (Schering-Plough, Kenilworth, NJ, USA), and amphotericin B (Bristol-Myers Squibb, Woerden, Netherlands) were determined according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document (Wayne PA., 2008). Antifungal agents including amphotericin B, itraconazole, voriconazole, posaconazole, and caspofungin were dispensed into the microdilution trays at final concentrations of 0.016–16 μg/ml. Inoculum suspensions were prepared on potato dextrose agar for 2-3 days by slightly scraping the surface of mature colonies with a sterile cotton swab, soaked in sterile saline solution containing Tween 40 (0.05%). The supernatants were adjusted spectrophotometrically to an optical density range of 0.09-0.13 (0.5×10⁴ to 3.1×10⁴ CFU/ml) at a wavelength of 530 nm, as determined by the quantitative colony count for specifying the viable CFUs per milliliter. Conidial suspensions, which mostly consisted of conidia, were diluted 1:50 in RPMI 1640 medium. The microdilution plates were inoculated with 100 μl of the diluted conidial inoculum suspension and incubated at 35°C for 48 h; the plates were read visually after agitation. Moreover, *Paecilomyces variotii* (ATCC 22319) and *Candida parapsilosis* (ATCC 22019) were used as the quality controls. With the aid of a reading mirror, MIC endpoints were determined as the...
lowest concentrations of drugs, inhibiting recognizable fungal growth (100% inhibition). Also, MECs were defined microscopically as the lowest concentrations of drugs, leading to the growth of small, rounded, and compact hyphal forms, compared to long, unbranched, hyphal clusters in the controls (Wayne PA., 2008). Considering the breakpoints proposed by Verweij et al. for itraconazole, voriconazole (susceptible: < 2 mg/L, intermediate: 2 mg/L, and resistant: > 2 mg/L), and posaconazole (susceptible: < 0.5 mg/L, intermediate: 0.5 mg/L, and resistant: > 0.5 mg/L) (Verweij et al., 2009a), itraconazole (1 mg/L), voriconazole (1 mg/L), and posaconazole (0.5 mg/L) with MICs above the proposed epidemiological cut-off values (ECVs) against A. fumigatus isolates were selected for further analysis (Espinel-Ingroff et al., 2010, Rodriguez-Tudela et al., 2008).

**DNA analysis of CYP51A gene**

Conventional polymerase chain reaction (PCR) assay was carried out to determine the presence of TR34/L98H mutation in the CYP51A gene of triazole-resistant A. fumigatus isolates (MIC> 2 µg/ml) in a total volume of 25 µl, containing 12.5 µl of Taq 2× Master Mix Red [Tris-HCl, pH=8.5, (NH4)2SO4, 4 mM MgCl2, 0.2% Tween 20, 0.4 mM deoxynucleotides, 0.2 units/µl of Ampliqon Taq DNA Polymerase (Ampliqon, Odense M; Denmark), inert red dye, and stabilizer], 10 pmol of AFCYPPF (5'-AATAATCGCAGCACCACCTTC-3) and AFCYPPR (5'TGGTATGCTGGAACTACACCTT-3) primers, 2 µl of template DNA, and 8.5 µl of distilled water.

PCR amplification started with an initial denaturation at 95°C for one min, followed by 35 cycles of denaturation at 94°C (60 s), 60°C (30 s), and 72°C (60 s) and a final 10 min extension at 72°C. PCR products were run on 2% agarose gel (Ahmad et al., 2014). Azole-resistant A. fumigatus isolates, containing TR34 in the CYP51A promoter region, were expected to yield a 139-bp amplicon, whereas the wild-type sequence (no tandem repeat) was speculated to yield a 105-bp amplicon (Ahmad et al., 2014).
For isolates with reduced susceptibility to itraconazole and voriconazole (MIC > 2 µg/ml), according to CLSI breakpoints (Verweij et al., 2009a), the whole CYP51A gene was amplified, using three pairs of primers (Chen J., 2005). The used primers were as follows: CYP1-L [5’-CACCCCTCCCTGTGTCTCCT], CYP1-R [5’-AGCCTTGAAAGTTCGGTGAA], CYP2-L [5’-CATGTGCCACTTATTGAGAAGG], CYP2-R [5’-CCTTGCAGATAGAGTGA], CYP3-L [5’-TTCCTCCGCTCCAGTACAG], and CYP3-R [5’-CCTTTGAAGTCCTCGATGGT] (Chen et al., 2005). Each fragment was sequenced from both ends. PCR conditions were similar to the conditions of tandem repeat amplification in the CYP51A promoter region. To detect possible mutations in the PCR products, which were assessed via DNA sequence analysis, the sequence of the products was compared with wild-type A. fumigatus CYP51A sequence, using MEGA software version 5 (Tamura et al., 2011).

**Mutation and microsatellite genotypic analysis**

All resistant A. fumigatus strains were subjected to mixed-format real-time PCR assay for the detection of mutations responsible for triazole resistance (Chowdhary et al., 2012). Genotyping of azole-resistant A. fumigatus and wild-type isolates was performed with a panel of nine short tandem repeats (Chowdhary et al., 2012). For phylogenetic analysis, clinical (n=3) and environmental (n=4) A. fumigates isolates (wild type) were used. Also, the resistant strains with or without mutations at TR34/L98H were both clinical (n=2) and environmental (n=6) (Figure 2).

**Statistical analysis**

The MIC values were calculated for clinical and environmental samples and the strains were compared. For statistical analysis, Chi-square test of homogeneity was performed at a significance level of 5% (Daniel, 1987).
Results

Based on the present three-year study, 513 specimens, consisting of clinical (n=213; 41.5%) and environmental (n=300; 58.4%) isolates, were obtained from ten provinces of Iran including Khorasan Razavi (n=120; 23.4%), Tehran (n=105; 20.5%), Mazandaran (n=98; 19.1%), West Azerbaijan (n=45; 8.7%), Shiraz (n=40; 7.8%), Hamadan (n=30; 5.8%), Ahvaz (n=20; 3.9%), Isfahan (n=20; 3.9%), Golestan (n=20; 3.9%), and East Azerbaijan (n=15; 2.9%) (Figure 1). The obtained isolates, which were initially identified as Aspergillus species, were confirmed via molecular assessments. Nucleotide sequences for the determined isolates were deposited in the GenBank database under accession numbers XXX to XXX (submitted to GenBank). The results showed that 95% of the isolates were 99–100% identical to β-tubulin genes of A. fumigatus. Finally, 150 A. fumigatus isolates (71 clinical and 79 environmental isolates) were confirmed.

Antifungal susceptibility tests

Table 1 summarizes the results of in vitro antifungal susceptibility tests, i.e., MIC range, geometric mean MIC, MIC50, and MIC90. Basically, the geometric mean MIC of posaconazole for all clinical and environmental strains was low (0.049 and 0.089 μg/ml, respectively); a similar finding was reported for the MEC of caspofungin (0.062 and 0.063 μg/ml, respectively). Remarkably, for clinical and environmental strains, the highest MICs in an increasing order were reported in voriconazole (0.085 and 0.241 μg/ml, respectively), itraconazole (0.519 and 0.742 μg/ml, respectively), and amphotericin B (0.567 and 0.707 μg/ml, respectively). Although clinical A. fumigatus isolates were more susceptible to azoles, compared to the environmental isolates, no statistically significant difference was detected (P>0.05). On the other hand, a significant difference was observed between the strains (P<0.05). According to in vitro antifungal susceptibility tests, MIC values above the clinical cut-off points against 10 (6.6%) strains, i.e., clinical (4.2%) and environmental (7.6%) strains
were reported in at least one of the antifungal agents (Table 2). Based on the findings, only
five (3.3%) strains were cross-resistant to itraconazole, voriconazole, and posaconazole. The
high MIC distributions of itraconazole and voriconazole were shifted approximately more
than two log₂ dilution steps apart.

**DNA analysis of CYP51A gene**

PCR amplification of TR34/L98H mutation in the CYP51A gene of resistant isolates (MIC > 2
µg/ml for itraconazole and voriconazole) yielded an amplicon of 139 bp (8:10; 80%), while
susceptible isolates (MIC < 2 µg/ml) yielded an amplicon of 105 bp (142:150). For
confirmation and observation of mutations in the entire CYP51A gene, DNA sequencing was
performed for resistant isolates (MIC > 2 µg/ml). It was confirmed that all resistant isolates
contained a CYP51A mutation. The only mutation found in eight (5.3%) isolates was
TR34/L98H (leucine-to-histidine substitution). In contrast, TR46/Y121F/T289A mutation was
not detected in the CYP51A promoter region (Table 2). Microsatellite analysis of all Iranian
itraconazole-resistant *A. fumigatus* isolates revealed a single TR34/L98H genotype. However,
resistant isolates were genetically different from their azole-susceptible counterparts (Figure
2).

**Discussion**

According to the European Centre for Disease Control (ECDC), the overall burden of
aspergillosis is estimated at 2.4 million cases each year in European countries. Azoles,
specifically voriconazole and itraconazole, are the recommended treatment options for such
infections (Kleinkauf, 2013). In recent years, clinical and environmental isolates of triazole-
resistant *A. fumigatus* have been reported in European countries including the Netherlands
(38%), the United Kingdom (20%), Italy (13%), Turkey (10.2%), France (8%), Denmark
(4.5%), Spain (4.2%), and Germany (3.2%). Similarly, the high prevalence of triazole-
resistant *A. fumigatus* has been revealed in other countries such as Australia (1%), China (5.8
According to previous research in Iran, the prevalence of clinical and environmental azole-resistant *A. fumigatus* isolates has been estimated at 3.2% and 3.3%, respectively (Badali et al., 2013, Seyedmousavi et al., 2013). In contrast with the present findings, in previous research, with the increased rate of azole resistance (4.2% and 7.6% for clinical and environmental *A. fumigatus* isolates, respectively), a 34-bp tandem repeat (TR34) sequence was observed in the CYP51A promoter region in combination with L98H substitution. The estimated rates were lower than the values reported in European countries and higher than the rates in Asian countries. Recently, Van der Linden et al. described a novel CYP51A-mediated resistance mechanism, consisting of two amino acid substitutions and a 46-bp tandem repeat in the TR46/Y121F/T289A gene promoter region (van der Linden et al., 2013). This mechanism could majorly reduce susceptibility to voriconazole (MIC> 16 µg/ml), while reducing resistance to itraconazole and posaconazole (MIC: 0.25-0.2 µg/ml). They revealed that 20.6% of patients harbored azole-resistant strains due to TR46/Y121F/T289A mutations. Moreover, this resistant mechanism has been reported in Belgium (Vermeulen et al., 2012), India (Chowdhary et al., 2013), Denmark (Astvad et al., 2014), and Germany (Fischer et al., 2014). However, in the present study, none of the isolates harbored TR46/Y121F/T289A, G54, M220, G138C, or G432C mutations. As discussed earlier, TR34/L98H resistance mechanism has been shown to have a vast geographical spread. Such isolates can be found in the environment, as well as azole-susceptible patients (Mortensen et al., 2010, Bader et al., 2013, Chowdhary et al., 2011). Interestingly, individuals with no prior use of azole antifungals may develop infections from the environment through frequent exposure of the fungus to azole fungicides used in agriculture. Lavergne and colleagues described the first azole-resistant *A. fumigatus* with TR46/Y121F/T289A mutations in a patient with cystic fibrosis in France where this mutation had not been previously.
reported. The patient had traveled to the Netherlands and regularly received advertising postal packages from Dutch flower companies. The researchers believed that inhalation and colonization of spores harboring TR_{46}/Y121F/T289A mutations were responsible for the patient’s poor condition. Also, flower packages from the Netherlands and the patient’s surroundings were speculated as the possible causes. In this case, French resistant strains were genetically distinguishable from Dutch isolates; it should be mentioned that the route of acquisition in this patient remained unknown (Lavergne et al., 2015). Notably, Chowdhary and colleagues reported an Indian azole-resistant *A. fumigatus* genotype, which was an extremely adaptive recombinant progeny. The significant phylogenetic incompatibility was consistent with the mating of natural populations of this species in India (Chowdhary et al., 2012); it seems that the spores followed an airborne migration. Medical application of azole antifungals seems to contribute to the widespread azole resistance in the environment. For instance, itraconazole, which is annually used in European countries, cannot be excreted from the human body in its active form, considering the high amount of agricultural azoles. In addition, shampoos, supplemented with 2% ketoconazole and fluconazole for the treatment of pityriasis versicolor or candidiasis, are unlikely to be effective, as the two components have shown negligible activities against *Aspergillus* species (Bowyer and Denning, 2014).

The present epidemiological study demonstrated that some isolates with TR_{34}/L98H mutations were highly resistant to itraconazole, while showing cross-resistance to voriconazole and posaconazole (5 out of 10, 50%). Nevertheless, we found a significant number of isolates for which no mutation in the *CYP51A* gene could be identified (2 out of 10, 20%). Therefore, other unrelated mechanisms such as increased production of drug target *CYP51A* protein (Arendrup et al., 2010, Camps et al., 2012, Albarrag et al., 2011) or overexpression of efflux pumps (Cannon et al., 2009, Manavathu et al., 1999, Bowyer et al., 2012, Slaven et al., 2002) should be considered. In conclusion, although the number of
patients affected by azole-resistant *A. fumigatus* isolates was limited, strict supervision of clinical azole-resistant *A. fumigatus* isolates and persistent environmental screening of azole resistance are vital to the development of approaches for the management of azole resistance in human pathogenic fungi.

**Acknowledgments**

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**Conflicts of interest**

There were no conflicts of interest in this study. The authors are responsible for the content and writing of the manuscript.
References:


Kleinkauf, N. 2013. Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in Aspergillus species, ECDC. 357


Legends

Figure 1. Distribution of Aspergillus fumigatus isolates in Iran. A total of 513 clinical and environmental isolates were obtained from ten provinces. Clinical and environmental azole-resistant A. fumigatus strains harboring TR34/L98H mutations are marked with red and green circles, respectively.

Figure 2. Genotypic relationship between eight resistant (TR34/L98H) and seven wild-type Aspergillus fumigatus isolates from Iran. The dendrogram is based on a categorical analysis of nine microsatellite markers in combination with unweighted pair group method with arithmetic mean clustering.

Table 1. In vitro antifungal susceptibility of 150 clinical and environmental Aspergillus fumigatus strains to five antifungal drugs. MIC range, geometric mean (GM), MIC50, and MIC90, expressed in mg/L.

Table 2. Characteristics of clinical and environmental A. fumigatus isolates with decreased drug susceptibility and mutations
Table 1. In vitro antifungal susceptibilities of a subset of 150 clinical and environmental strains of *Aspergillus fumigatus* to five antifungal drugs. MIC range, Geometric mean (GM), MIC\(_{50}\) and MIC\(_{90}\) values expressed in mg/L.

| Source of origin          | Number | Antifungal agent | 0.008 | 0.016 | 0.031 | 0.063 | 0.125 | 0.25 | 0.5 | 1    | 2    | 4    | 8    | >16   | MIC range | MIC \(_{50}\) | MIC \(_{90}\) | GM     |
|--------------------------|--------|------------------|-------|-------|-------|-------|-------|------|-----|------|------|------|------|--------|-----------|-----------|---------|
| All *A. fumigatus*       | 71     | AMB              | -     | -     | -     | -     | 9     | 13   | 35  | 12   | 1    | 1    | -    | -      | 0.125–4   | 0.5       | 0.5     | 0.5674 |
| (Clinical strains)       |        | ITC              | -     | -     | -     | 7     | 15    | 13   | 25  | 6    | 2    | 1    | 2    | 0.5    | 0.063–>16 | 0.5       | 0.5     | 0.5196 |
|                          |        | VRC              | -     | -     | 3     | 23    | 20    | 15   | 7   | 1    | 1    | 1    | -    | 0.031–8 | 0.063     | 0.25      | 0.0855  |
|                          |        | POS              | 7     | 2     | 20    | 21    | 13    | 6    | -   | -    | 1    | 1    | -    | 0.008–4 | 0.063     | 0.25      | 0.0491  |
|                          |        | CAS              | 14    | 9     | 18    | 9     | 14    | 3    | 4   | -    | -    | -    | -    | 0.008–0.5 | 0.063     | 0.125     | 0.0622  |
| All *A. fumigatus*       | 79     | AMB              | -     | -     | -     | -     | 8     | 12   | 30  | 23   | 6    | -    | -    | 0.125–2 | 0.5       | 1        | 0.7071  |
| (Environmental strains)  |        | ITC              | -     | -     | 4     | 7     | 12    | 33   | 15  | 1    | 1    | 6    | -    | 0.063–>16 | 0.5       | 0.5     | 0.4426  |
|                          |        | VRC              | -     | -     | 1     | 8     | 22    | 24   | 16  | 4    | 1    | 2    | 1    | 0.031–>16 | 0.25      | 0.5     | 0.2414  |
|                          |        | POS              | 3     | 3     | 13    | 25    | 17    | 16   | -   | 2    | -    | -    | -    | 0.008–2 | 0.063     | 0.25      | 0.0869  |
|                          |        | CAS              | 4     | 3     | 22    | 21    | 26    | 2    | 1   | -    | -    | -    | -    | 0.008–0.5 | 0.063     | 0.125     | 0.0635  |

MIC\(_{50}\) concentration at which 50% of the isolates were inhibited, MIC\(_{90}\) concentration at which 90% of the isolates were inhibited, MEC minimum effective concentrations; AMB (amphotericin B), ITC (itraconazole), VRC (voriconazole), POS (posaconazole), CAS (caspofungin)
Table 2. Characteristics of clinical and environmental *Aspergillus fumigatus* isolates with decreased drug susceptibility and mutations

<table>
<thead>
<tr>
<th>Number</th>
<th>Isolate Name</th>
<th>Source of isolate</th>
<th>City</th>
<th>MIC range (mg/L)</th>
<th>CYP51A Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITC VRC POS CAS AMB TR34/ L98H TR46/ Y121F G54 M220 G186C T289A Y121F G432C</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>IFRC 441</td>
<td>Clinical (bronchoalveolar lavage)</td>
<td>Tehran</td>
<td>&gt;16 4 4 0.25 1</td>
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<td>2</td>
<td>IFRC 442</td>
<td>Environmental (hospital soil)</td>
<td>Tehran</td>
<td>&gt;16 4 2 0.125 1</td>
<td>+ - - - - - - - - - -</td>
</tr>
<tr>
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<td>Sari</td>
<td>&gt;16 8 2 0.008 0.25</td>
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<td>Environmental (hospital soil)</td>
<td>Tehran</td>
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<td>+ - - - - - - - - - -</td>
</tr>
<tr>
<td>5</td>
<td>IFRC 1032</td>
<td>Environmental (hospital airflow)</td>
<td>Mashhad</td>
<td>&gt;16 8 0.016 0.031 0.5</td>
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<td>6</td>
<td>IFRC 435</td>
<td>Environmental (hospital soil)</td>
<td>Tehran</td>
<td>&gt;16 8 2 0.016 0.5</td>
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<td>7</td>
<td>IFRC 1000</td>
<td>Environmental (hospital airflow)</td>
<td>Mashhad</td>
<td>&gt;16 1 0.25 0.125 0.5</td>
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<td>Ahvaz</td>
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<td>9</td>
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<td>Clinical (bronchoalveolar lavage)</td>
<td>Sari</td>
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Abbreviations: IFRC: Invasive Fungi Research Center, Sari, Iran