Review

Current Status of Azole-resistant *Aspergillus fumigatus* Isolates in East Asia: China, Japan, Korea, and Taiwan

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ABSTRACT

*Aspergillus fumigatus* is a saprophytic fungus that is a major causative pathogen for aspergillosis. Only a few classes of antifungals are used for treating this life-threatening fungal infection. Azoles are the first-line drugs and are widely used for the management and prophylaxis of aspergillosis. An emerging issue is the increasing incidence of resistant isolates worldwide. In particular, environmentally derived tandem-repeat-type azole-resistant mutations, such as Cyp51A TR34/L98H, and Cyp51A TR46/Y121F/T289A, have emerged over the last decade. In particular, azole-resistant isolates were prevalent in clinical settings in European countries; many of the reports are from the Netherlands, UK, and Germany. In contrast, reports on azole-resistant *A. fumigatus* isolates from East Asian countries are still few and have only recently begun to increase. Herein, all literature on East Asian azole-resistant *A. fumigatus* isolates were reviewed, and a complete list of resistant isolates from China, Japan, Taiwan, and Korea is provided. As of this report, the total numbers of tandem-repeat-type azole-resistant isolates are 26, 3, 32, and 1 in China, Japan, Taiwan, and Korea, respectively.

Key words: *Aspergillus fumigatus*, azole resistance, Cyp51A, mutation

Introduction

Aspergillosis is a life-threatening fungal infection caused by *Aspergillus* species. The main etiological species is *Aspergillus fumigatus*, which is ubiquitous in the environment and has conidia that can be easily dispersed into the air<sup>1</sup>. Although humans inhale the conidia every day, the immune system prevents infection from developing. Mainly infected are immunocompromised patients, such as hematopoietic stem cell transplantation recipients, and at high mortality. There are only a few antifungals on hand to combat the causative fungi. The most effective therapy recommended for aspergillosis is azole drugs, which target the Cyp51A protein, a sterol 14 α-demethylase. Currently, itraconazole (ITCZ), voriconazole (VRCZ), and posaconazole (POS) are used for aspergillosis therapy.

In the last decade, azole drug resistance has gained wider attention worldwide. Since the itraconazole-resistant *A. fumigatus* was first reported in 1997<sup>2</sup>, the prevalence of azole-resistant isolates has apparently increased to the extent that first-line azole therapy is now in danger of becoming ineffective<sup>3-6</sup>. The mechanism for the occurrence of azole resistance is largely two-fold<sup>5</sup>. 1) During prolonged drug treatment, pathogens are exposed to the drugs in the patient’s body and occasionally evolve to overcome the inhibition; specifically, by acquiring resistant mutations. 2) Outside the patient’s body, pathogens can encounter a moderate concentration of azoles. In particular, the most plausible assumption is that widely used azole fungicides (DMIs: demethylase inhibitors) induce the resistance mutation in *A. fumigatus* in the environment<sup>6</sup>. The mutation can then cause cross-resistance to medically used azole drugs.

There are several epidemiological studies and case reports on azole-resistant *A. fumigatus*, and the mechanism of azole resistance mutations has been the subject of many researches<sup>5</sup>. For example, regarding in-host evolution, G54, M220, and G448 in the Cyp51A are hotspots for azole resistance mutation, whereas tandem repeats (TR34 and TR46) in the cyp51A gene promoter, with a combination of some amino acid substitutions (L98H and Y121F/T289A), are the environmentally derived resistance mutations. According to...
recent studies from the Netherlands, the environmentally developed azole resistance mutation is becoming the most frequent type of mutation\(^7\). Isolates with azole resistance may already be present in nature and are able to survive even in niches containing DMIs. This suggests a possible enrichment of the azole resistant \textit{A. fumigatus} in the environment. Compared with European countries, environmental azole resistance mutations were isolated more recently in East Asian countries, such as China, Japan, Korea, and Taiwan\(^8-11\)). Thus, compiling data on the azole-resistant isolates from these East Asian countries is a timely issue, which will provide useful information for further study and diagnosis in clinical settings. We therefore conducted a search in PubMed (9th Jul, 2018) for all reports from countries where azole-resistant isolates in \textit{A. fumigatus} and its mutation in Cyp51A had been identified.

### Azole-resistant \textit{A. fumigatus} isolates in China

The first isolation of azole-resistant \textit{A. fumigatus} in an East Asian country was reported in 2005\(^17\). The strains were serially isolated from a patient with aspergillosis between 2002 and 2003 in Beijing. ITCZ was used for the therapy and two azole-resistant mutations in Cyp51A were identified; namely, M220I and G54R (Table 1). From China, azole-resistant isolates with a tandem-repeat mutation were first identified from the ARTEMIS global surveillance study published in 2011\(^18\). The mutation included S297T and F495I, as well as TR34 and L98H, which were found to be prevalent in China, whereas they were reported in only a few studies from Europe. The other type of tandem-repeat mutant (TR46, Y121F, T289A) appeared between 2010 and 2015\(^19\), wherein strains with Cyp51A TR34/L98H/S297T/F495I were isolated from the environment.

### Azole-resistant \textit{A. fumigatus} isolates in Japan

Although the papers were published in 2011 and 2012, the azole-resistant isolates had been found in Japan as early as around 2000 (1998-2002)\(^17,18\)). The mutations in these isolates were amino acid substitutions rather than tandem-repeat-type mutations. Most of the isolates harbored the mutation at G54, which is a hotspot site in \textit{A. fumigatus} Cyp51A with variable substitutions (G54E, G54W, and G54R) (Table 2). The VRCZ-resistant strains were isolated from patients with a history of VRCZ treatment; all of these isolates had the G448S mutation \(^20\). The first azole-resistant strain with a tandem-repeat mutation (TR46/Y121F/T289F) in Japan was clinically isolated in 2013\(^20\), followed by isolation of the TR34/L98H strain (OKH50) in 2016\(^19\). The TR34/L98H strain (Env1) was also isolated from the environment in Obihiro, in the northern part of Japan\(^22\). Notably, the two isolates (OKH50 and Env1) from Obihiro shared identical STRs, indicating a clonal relatedness between clinical and environmental azole-resistant isolates. In the most recent work by Hagiwara et al.\(^20\), a set of non-cyp51A azole-resistant \textit{A. fumigatus} isolates was reported, some of which had a mutation in the \textit{hmg1} gene encoding a HMG-CoA reductase. This suggests that Hmg1 is involved in azole resistance.

### Azole-resistant \textit{A. fumigatus} isolates in Korea and Taiwan

A handful of clinically isolated azole-resistant isolates were reported from Taiwan\(^27\). These were either TR34/L98H or TR34/L98H/S297T/F495I, and they all showed ITCZ resistance. In contrast, in-host evolved resistant isolates have not been reported so far. In the nationwide environmental surveillance study by Wang HC et al.\(^29\), many resistant strains with either TR34/L98H (n = 12) or TR34/L98H/S297T/F495I (n = 17) were identified from the environment. The strains isolated in different areas of Taiwan showed identical STR genotypes, indicating that clonal mutant strains are widely distributed. Intriguingly, some environmental isolates had identical STR genotypes to the clinically isolated strain previously identified by Wu et al.\(^27\). These results suggest that such tandem-repeat-type resistant isolates are prevalent throughout the country.

In Korea, only one azole-resistant clinical \textit{A. fumigatus} was recently reported\(^20\). This isolate had the TR34/L98H/S297T/F495I mutation.

### Concluding remarks

Azole-resistant \textit{A. fumigatus} strains have been continuously isolated in East Asian countries. It seems that the number of tandem-repeat-type resistant isolates is increasing. They are, to some extent, already present in the soil and air in the study sites. After their emergence, it is almost impossible to eliminate them from the environment, and they could further increase in the future. We are now beyond the point of no return, which signifies the need for more careful monitoring of such environmental azole-resistant isolates, more investment in developing novel antifungals, and simpler and more reliable diagnostic innovations.

### Acknowledgments

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### Conflicts of interest

None.
Table 1. List of azole-resistant *A. fumigatus* isolates from China

| Isolate ID | Year of isolation | Region                | Source | Resistant to: *2 | Mutation in Cyp51A | References |
|------------|-------------------|-----------------------|--------|------------------|---------------------|------------|
| AF2        | 2002              | Beijing               | Clinical | ITCZ             | M220I               | 12)        |
| AF4        | 2002              | Beijing               | Clinical | ITCZ             | G54R                | 12)        |
| AF5        | 2003              | Beijing               | Clinical | ITCZ             | G54R                | 12)        |
| AF6        | 2003              | Beijing               | Clinical | ITCZ             | G54R                | 12)        |
| 20643.017  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20643.023  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20677.079  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20677.086  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20677.089  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, VRCZ, POS  | TR34, L98H, S297T, F495I | 8)        |
| 20684.002  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20684.003  | (2008-2009)       | Not shown             | Clinical | ITCZ, POS        | -                   | 8)        |
| 20684.007  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20684.022  | (2008-2009)       | Hangzhou              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 8)        |
| 20684.015  | (2008-2009)       | Not shown             | Clinical | ITCZ, POS        | -                   | 8)        |
| 20684.006  | (2008-2009)       | Not shown             | Clinical | POS              | -                   | 8)        |
| Shhs18     | (2011-2014)       | Shanghai              | Clinical | ITCZ             | G432A               | 14)        |
| Shjh40     | (2011-2014)       | Shanghai              | Clinical | ITCZ             | TR34, L98H          | 14)        |
| Shjh42b    | (2011-2014)       | Fuzhou                | Clinical | ITCZ             | TR34, L98H, S297T, F495I | 14)        |
| Nj21-76    | (2011-2014)       | Nanjing              | Clinical | ITCZ             | TR34, L98H, S297T, F495I | 14)        |
| C94        | (2010-2015)       | Shanghai              | Clinical | ITCZ, POS        | TR34, L98H          | 13)        |
| C96        | (2010-2015)       | Shanghai              | Clinical | ITCZ             | TR34, L98H, S297T, F495I | 13)        |
| C116       | (2010-2015)       | Fuzhou                | Clinical | ITCZ, VRCZ       | TR34, L98H          | 13)        |
| C135       | (2010-2015)       | Fuzhou                | Clinical | ITCZ             | TR34, L98H          | 13)        |
| C136       | (2010-2015)       | Fuzhou                | Clinical | ITCZ             | TR34, L98H          | 13)        |
| C195       | (2010-2015)       | Beijing               | Clinical | VRCZ             | TR46, Y121F, T289A  | 13)        |
| C485       | (2010-2015)       | Shenyang              | Clinical | ITCZ, POS        | TR34, L98H, S297T, F495I | 13)        |
| E739       | (2010-2015)       | Beijing               | Environment | ITCZ             | TR34, L98H, S297T, F495I | 13)        |
| C821       | (2010-2015)       | Chengdu               | Clinical | ITCZ, VRCZ, POS  | TR34, L98H          | 13)        |
| E1001      | (2010-2015)       | Fuzhou                | Environment | ITCZ             | TR34, L98H, S297T, F495I | 13)        |
| No. 15     | Not shown         | Hangzhou              | Environment | VRCZ             | TR46, Y121F, T289A  | 15)        |
| No. 44     | Not shown         | Fuyang                | Environment | VRCZ             | TR46, Y121F, T289A  | 15)        |
| No. 51     | Not shown         | Jiande                | Environment | ITCZ             | TR34, L98H, S297T, F495I | 15)        |
| AF.28      | (2012-2015)       | Nanjing              | Clinical | ITCZ             | M220I               | 16)        |
| AF.44      | (2012-2015)       | Nanjing              | Clinical | ITCZ, VRCZ       | TR34, L98H          | 16)        |
| AF.98      | (2012-2015)       | Nanjing              | Clinical | ITCZ             | TR34, L98H          | 16)        |
| AF.118     | (2012-2015)       | Nanjing              | Clinical | ITCZ             | -                   | 16)        |

*1: Period when the study was conducted

*2: Resistance is regarded as ITCZ (> 2), VRCZ (> 2), POS (> 0.5)

*3: EUCAST protocol was used.
### Table 2. List of azole-resistant *A. fumigatus* isolates from Japan

| Isolate ID | Year of isolation | Region         | Source | Resistant to *1 | Mutation in Cyp51A | References |
|------------|-------------------|----------------|--------|-----------------|--------------------|------------|
| 2906       | 2000              | Not shown      | Clinical | ITCZ *2         | n.d. *4            | 17)        |
| 5402       | 2000              | Not shown      | Clinical | ITCZ *2         | V119L, N248K, K256Q | 17)        |
| MF-452     | 2002              | Nagasaki       | Clinical | ITCZ            | I266N              | 18)        |
| MF-460     | 2002              | Nagasaki       | Clinical | ITCZ, POS       | G54E, I266N        | 18)        |
| MF-468     | 2002              | Nagasaki       | Clinical | ITCZ            | G54E, I266N        | 18)        |
| MF-469     | 2002              | Nagasaki       | Clinical | ITCZ            | G54E, I266N        | 18)        |
| MF-329     | 1998              | Nagasaki       | Clinical | ITCZ            | -                  | 18)        |
| MF-331     | 1998              | Nagasaki       | Clinical | POS             | G54W               | 18)        |
| MF-357     | 2000              | Nagasaki       | Clinical | ITCZ            | -                  | 18)        |
| MF-1011    | 2008              | Nagasaki       | Clinical | POS             | G54W               | 18)        |
| MF-327     | 1998              | Nagasaki       | Clinical | POS             | G54R               | 18)        |
| IFM 50233  | 2000              | Not shown      | Clinical | ITCZ, POS       | G54E, N248K        | 23)        |
| IFM 60237  | 2011              | Chiba          | Clinical | ITCZ            | P216L              | 24)        |
| IFM 63432  | 2013              | Tokyo          | Clinical | VRCZ            | TR46, Y121F, T289A | 9)         |
| OKH34      | 2015              | Obihiro        | Clinical | VRCZ, POS       | G448S              | 19)        |
| IFM 61567  | 2011              | Iwate          | Clinical | ITCZ            | G54E               | 25)        |
| OKH50      | 2016              | Obihiro        | Clinical | ITCZ, VRCZ, POS | TR34, L98H         | 21)        |
| Env1       | 2016              | Obihiro        | Environment | ITCZ, VRCZ, POS | TR34, L98H         | 22)        |
| NIH0345    | 2016              | Aichi          | Clinical | ITCZ *2         | G138S, N248L       | 26)        |
| IFM 62916  | 2014              | Chiba          | Clinical | ITCZ, VRCZ, POS | G448S *7           | 20)        |
| IFM 63240  | 2014              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63241  | 2014              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63242  | 2014              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63243  | 2014              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63249  | 2015              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63537  | 2015              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63594  | 2015              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63595  | 2015              | Chiba          | Clinical | ITCZ, VRCZ, POS | G448S *7           | 20)        |
| IFM 63596  | 2015              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63714  | 2015              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 64173  | 2016              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 62140  | 2013              | Kanagawa       | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 64258  | 2016              | Ibaraki        | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 64303  | 2016              | Tokyo          | Clinical | VRCZ            | -                  | 20)        |
| IFM 63666  | 2015              | Chiba          | Clinical | POS             | -                  | 20)        |
| IFM 63768  | 2016              | Chiba          | Clinical | ITCZ, VRCZ, POS | - *7               | 20)        |
| IFM 63772  | 2016              | Chiba          | Clinical | VRCZ, POS       | -                  | 20)        |

*1: ITCZ (> 2), VRCZ (> 2), POS (> 0.5)

*2: E-test for MIC

*3: MIC for POS is tested elsewhere.

*4: n.d. (not determined)

*5: Only partially sequenced (68-336)

*6: F332K was described in the reference, but was not found in the sequence deposited in DDBJ.

*7: Has mutation in *hmg1* gene
Table 3. List of azole-resistant *A. fumigatus* isolates from Taiwan and Korea

| Isolate ID | Year of isolation | Region | Source | Resistant to: *2 | Mutation in Cyp51A | References |
|------------|-------------------|--------|--------|------------------|---------------------|------------|
| A31        | (2011-2014) *1    | Southern Taiwan | Clinical | ITCZ, VRCZ, POS | TR34, L98H | 27) |
| B44        | (2011-2014) *1    | Northern Taiwan | Clinical | ITCZ, POS | TR34, L98H, S297T, F495I | 27) |
| B51        | (2011-2014) *1    | Northern Taiwan | Clinical | ITCZ, POS | TR34, L98H, S297T, F495I | 27) |
| *A. fumigatus* (n = 12) | (2014-2016) *1 | Taiwan | Environment | ITCZ, POS, (VRCZ) | *3 | TR34, L98H | 28) |
| *A. fumigatus* (n = 17) | (2014-2016) *1 | Taiwan | Environment | ITCZ, POS, (VRCZ) | *4 | TR34, L98H, S297T, F495I | 28) |
| SD359      | (2014-2016) *1    | Taiwan | Environment | ITCZ, VRCZ, POS | I242V | 28) |
| SD116A3    | (2014-2016) *1    | Taiwan | Environment | VRCZ | - | 28) |
| TN061-2    | (2014-2016) *1    | Taiwan | Environment | ITCZ, VRCZ | - | 28) |
| *A. fumigatus* (n = 1) | Not shown | Korea | Clinical | ITCZ, POS | TR34, L98H, S297T, F495L | 11) |

*1: Period when the study was conducted
*2: ITCZ (> 2), VRCZ (> 2), POS (> 0.5)
*3: MIC for VRCZ ranged 2-4
*4: MIC for VRCZ ranged 1-4

References

1) Kwon-Chung KJ, Sugui JA: *Aspergillus fumigatus*—what makes the species a ubiquitous human fungal pathogen? PLoS Pathog 9: e1003743, 2013.
2) Denning DW, Venkateswarlu K, Oakley KL, Anderson MJ, Manning NJ, Stevens DA, Warnock DW, Kelly SL: Itraconazole resistance in *Aspergillus fumigatus*. Antimicrob Agents Chemother 41: 1364-1368, 1997.
3) Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC, Laverdiere M, Arendrup MC, Perlin DS, Denning DW: Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg Infect Dis 15: 1068-1076, 2009.
4) van der Linden JW, Arendrup MC, Warris A, et al: Prospective multicenter international surveillance of azole resistance in *Aspergillus fumigatus*. Emerg Infect Dis 21: 1041-1044, 2015.
5) Hagiwara D, Watanabe A, Kamei K, Goldman GH: Epidemiological and genomic landscape of azole resistance mechanisms in *Aspergillus* fungi. Front Microbiol 7: 1382, 2016.
6) Chowdhary A, Kathuria S, Xu J, Meis JF: Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. PLoS Pathog 9: e1003633, 2013.
7) van Ingen J, van der Lee HA, Rijs TA, Zoll J, Leenstra T, Melchers WJ, Verweij PE: Azole, polyene and echinocandin MIC distributions for wild-type, TR34/L98H and TR46/Y121F/T289A *Aspergillus fumigatus* isolates in the Netherlands. J Antimicrob Chemother 70: 178-181, 2015.
8) Lockhart SR, Frade JP, Etienne KA, Pfaller MA, Diekema DJ, Balajee SA: Azole resistance in *Aspergillus fumigatus* isolates from the ARTEMIS global surveillance study is primarily due to the TR/L98H mutation in the cyp51A gene. Antimicrob Agents Chemother 55: 4465-4468, 2011.
9) Hagiwara D, Takahashi H, Fujimoto M, Sugahara M, Misawa Y, Gono T, Itoyama S, Watanabe A, Kamei K: Multi-azole resistant *Aspergillus fumigatus* harboring Cyp51A TR46/Y121F/T289A isolated in Japan. J Infect Chemother 22: 577-579, 2016.
10) Wu CJ, Wang HC, Lee JC, Lo HJ, Dai CT, Chou PH, Ko WC, Chen YC: Azole-resistant *Aspergillus fumigatus* isolates carrying TR 34/L98H mutations in Taiwan. Mycoses 58: 544-549, 2015.
11) Lee HJ, Cho SY, Lee DG, Park C, Chun HS, Park YJ: TR 34/L98H mutation in CYP51A gene in *Aspergillus fumigatus* clinical isolates during posaconazole prophylaxis: First case in Korea. Mycopathologia 183: 731-736, 2018.
12) Chen J, Li H, Li R, Bu D, Wan Z: Mutations in the cyp51A gene and susceptibility to itraconazole in *Aspergillus fumigatus* serially isolated from a patient with lung aspergillosis. J Antimicrob Chemother 55: 31-37, 2005.
13) Chen Y, Lu Z, Zhao J, et al: Epidemiology and molecular characterizations of azole resistance in clinical and environmental *Aspergillus fumigatus* isolates from China. Antimicrob Agents Chemother 60: 5878-5884, 2016.
14) Liu M, Zeng R, Zhang L, Li D, Lv G, Shen Y, Zheng H, Zhang Q, Zhao J, Zheng N, Liu W: Multiple cyp51A-based mechanisms identified in azole-resistant isolates of *Aspergillus fumigatus* from China. Antimicrob Agents Chemother 59: 4321-4325, 2015.
15) Ren J, Jin X, Zhang Q, Zheng Y, Lin D, Yu Y: Fungicides induced triazole-resistance in *Aspergillus fumigatus* associated with mutations of TR46/Y121F/T289A and its appearance in agricultural fields. J Hazard Mater 326: 54-60, 2017.
16) Zhang M, Feng CL, Chen F, He Q, Su X, Shi Y: Triazole resistance in *Aspergillus fumigatus* clinical isolates obtained in
17) Asano M, Kano R, Makimura K, Hasegawa A, Kamata H: Molecular typing and in-vitro activity of azoles against clinical isolates of *Aspergillus fumigatus* and *A. niger* in Japan. J Infect Chemother 17: 483-486, 2011.

18) Tashiro M, Izumikawa K, Hirano K, et al: Correlation between triazole treatment history and susceptibility in clinically isolated *Aspergillus fumigatus*. Antimicrob Agents Chemother 56: 4870-4875, 2012.

19) Toyotome T, Fujiwara T, Kida H, Matsumoto M, Wada T, Komatsu R: Azole susceptibility in clinical and environmental isolates of *Aspergillus fumigatus* from eastern Hokkaido, Japan. J Infect Chemother 22: 648-650, 2016.

20) Hagiwara D, Arai T, Takahashi H, Kusuya Y, Watanabe A, Kamei K: Clinical isolates of non-*cyp51A* azole-resistant *Aspergillus fumigatus* harboring a mutation in *hmg1* gene encoding HMG-CoA reductase. Emerg Infect Dis (in press)

21) Toyotome T, Hagiwara D, Kida H, Ogi T, Watanabe A, Wada T, Komatsu R, Kamei K: First clinical isolation report of azole-resistant *Aspergillus fumigatus* with TR₁₄/L98H-type mutation in Japan. J Infect Chemother 23: 579-581, 2017.

22) Onishi K, Muhammad Sarumoh B, Hagiwara D, Watanabe A, Kamei K, Toyotome T: Azole-resistant *Aspergillus fumigatus* containing a 34-bp tandem repeat in *cyp51A* promoter is isolated from the environment in Japan. Med Mycol 58: E67-E70, 2017.

23) Kikuchi K, Watanabe A, Ito J, Oku Y, Wuren T, Taguchi H, Yarita K, Muraosa Y, Yahiro M, Yaguchi T, Kamei K: Antifungal susceptibility of *Aspergillus fumigatus* clinical isolates collected from various areas in Japan. J Infect Chemother 20: 336-338, 2014.

24) Hagiwara D, Takahashi H, Watanabe A, Takahashi-Nakaguchi A, Kawamoto S, Kamei K, Gonoi T: Whole-genome comparison of *Aspergillus fumigatus* strains serially isolated from patients with aspergillosis. J Clin Microbiol 52: 4202-4209, 2014.

25) Hagiwara D, Miura D, Shimizu K, Paul S, Ohba A, Gonoi T, Watanabe A, Kamei K, Shintani T, Toyoe-Rowley WS, Kawamoto S, Gomi K: A Novel Zn2-Cys6 transcription factor AtrR plays a key role in an azole resistance mechanism of *Aspergillus fumigatus* by co-regulating *cyp51A* and *cdr1B* expressions. PLoS Pathog 13: e1006096, 2017.

26) Umeyama T, Hayashi Y, Shimosaka H, Inukai T, Yamagoe S, Takatsuka S, Hoshino Y, Nagi M, Nakamura S, Kamei K, Ogawa K, Miyazaki Y: CRISPR/Cas9 genome editing to demonstrate the contribution of Cyp51A Gly138Ser to azole resistance in *Aspergillus fumigatus*. Antimicrob Agents Chemother 62: e00894-18, 2018.

27) Wu CJ, Wang HC, Lee JC, Lo HJ, Dai CT, Chou PH, Ko WC, Chen YC: Azole-resistant *Aspergillus fumigatus* isolates carrying TR₁₄/L98H mutations in Taiwan. Mycoses 58: 544-549, 2015.

28) Wang HC, Huang JC, Lin YH, Chen YH, Hsieh MI, Choi PC, Lo HJ, Liu WL, Hsu CS, Shih HI, Wu CJ, Chen YC: Prevalence, mechanisms and genetic relatedness of the human pathogenic fungus *Aspergillus fumigatus* exhibiting resistance to medical azoles in the environment of Taiwan. Environ Microbiol 20: 270-280, 2018.