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Multicenter Study of Azole-Resistant *Aspergillus fumigatus* Clinical Isolates, Taiwan[1]

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In a multicenter study, we determined a prevalence rate of 4% for azole-resistant *Aspergillus fumigatus* in Taiwan. Resistance emerged mainly from the environment (TR_{34}/L98H, TR_{34}/L98H/S297T/F495I, and TR_{46}/Y121F/T289A mutations) but occasionally during azole treatment. A high mortality rate observed for azole-resistant aspergillosis necessitates diagnostic stewardship in healthcare and antifungal stewardship in the environment.

Worldwide emergence of azole-resistant *Aspergillus fumigatus* since the late 2000s threatens human health (*1*). Azole resistance in *A. fumigatus* might develop during patient therapy with medical azoles or through exposure to azole fungicides in the environment; environmental exposure predominantly involves TR_{34}/L98H and TR_{46}/Y121F/T289A mutations in *cyp51A* (*1*).

Taiwan is an island country in eastern Asia that is geographically separated from mainland Eurasia and has a long history of azole fungicide use. To delineate the influence of clinical and environmental use of azoles on resistance, we conducted a multicenter study that investigated 375 *A. fumigatus* sensu stricto isolates collected during August 2011–March 2018 from 297 patients at 11 hospitals in Taiwan (Appendix Table 1, Figure 1, https://wwwnc.cdc.gov/EID/article/26/4/19-0840-App1.pdf).

We confirmed the presence of azole resistance by using the Clinical Laboratory Standard Institute method (Appendix Table 1) (*2*). Isolates resistant to ≥1 medical azoles (itraconazole, voriconazole, posaconazole, and isavuconazole) were defined as azole-resistant *A. fumigatus* and examined for resistance mechanisms, microsatellite-based phylogenetic relatedness, and growth rates following previously described methods (*3*,*4*).

Overall, 19 isolates from 12 patients were azole-resistant *A. fumigatus*. These isolates had resistance rates of 4.0%/patient and 5.1%/isolate analyses (Appendix Tables 2, 3). Ten (83.3%) patients harbored azole-resistant *A. fumigatus* that had environmental mutations, including TR_{34}/L98H (5 isolates, 5 patients), TR_{34}/L98H/S297T/F495I (7 isolates, 4 patients), and TR_{46}/Y121F/T289A (1 isolate) mutations. This observation

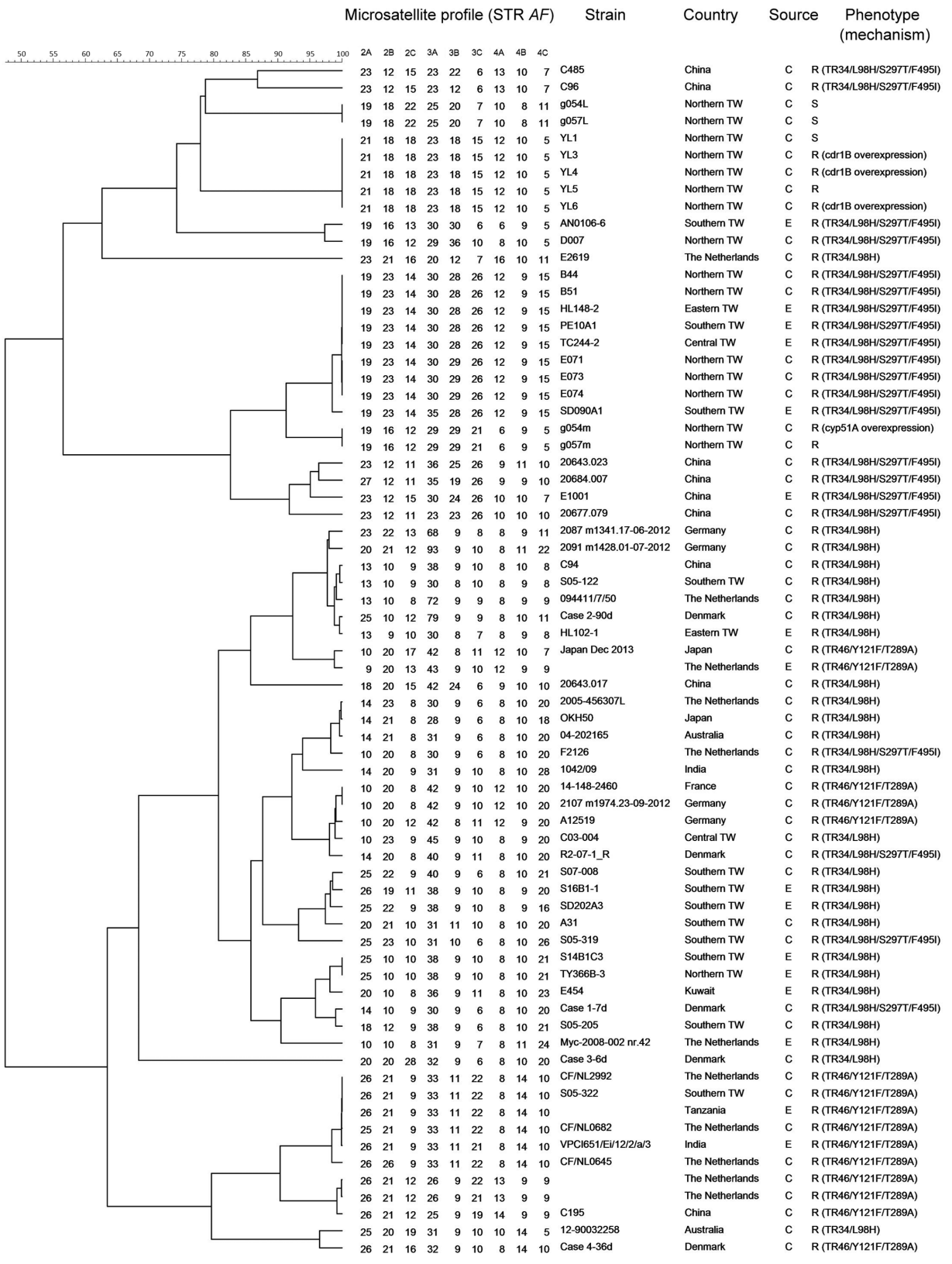

Figure. Genetic relatedness among *Aspergillus fumigatus* isolates based on microsatellite genotyping, Taiwan. Scale bar indicates percentage relatedness. *AF*, *A. fumigatus*; C, clinical; E, environmental; R, azole-resistant; S, azole-susceptible; STR, short tandem repeat; TW, Taiwan.

is consistent with the estimated global prevalence of azole resistance in *Aspergillus* (3%–6%) and the predominance of environmental resistance mechanisms in azole-resistant *A. fumigatus* (*1*,*5*).

Phylogenetic analysis showed that TR_{34}/L98H/S297T/F495I isolates from 2 patients with pulmonary aspergillosis (isolates B44 and B51 in 2012, isolates E071, E073, and E074 in 2015) (Figure) belonged to a local microsatellite genotype widely distributed in the environment of Taiwan (*3*), indicating that this clone has locally evolved and adapted to the environment. The TR_{34}/L98H isolates were genetically clustered with local environmental isolates or clinical isolates from China and Europe (Appendix Table 4). The TR_{46}/Y121F/T289A isolate (S05–322) recovered in 2018, which colonized a patient without overseas travel, was genetically identical to a clone prevalent in the Netherlands and Tanzania (*6*), raising the concern of the intercountry transfer of resistant isolates.

All TR_{34}/L98H/S297T/F495I, TR_{34}/L98H, and TR_{46}/Y121F/T289A isolates exhibited cross-resistance to difenoconazole and tebuconazole (both triazole fungicides) without fitness cost, demonstrated by normal growth rates (Appendix Figure 2). The TR_{34}/L98H/S297T/F495I isolates and TR_{46}/Y121F/T289A isolates were also resistant to prochloraz (an imidazole fungicide) (Appendix Table 2). The prevalence of TR_{34}/L98H/S297T/F495I isolates in Taiwan might be attributed to widespread use of prochloraz over the past 3 decades. Studies have suggested an association between use of imidazole fungicides and emergence of azole-resistant *A. fumigatus* with TR_{34}/L98H/S297T/F495I mutations (*7*,*8*).

In Taiwan, the annual consumption of difenoconazole and tebuconazole has exceeded that of prochloraz since 2012 (Appendix Figure 3), further creating a favorable environment for maintenance and spread of TR_{34}/L98H, TR_{34}/L98H/S297T/F495I, and TR_{46}/Y121F/T289A isolates. Thus, the One Health approach to implement environmental antifungal stewardship is warranted to minimize ongoing resistance selection in the fields.

Six azole-resistant *A. fumigatus* isolates with wild-type *cyp51A* were obtained from 2 patients. Four pan–azole-resistant urinary isolates were sequentially recovered from a patient (no. 11) with *A. fumigatus* renal abscesses who was receiving voriconazole for >3 months in whom an initial urine isolate was susceptible to azole; all 5 isolates were genetically identical.

Overexpression of *cdr1B* (a drug efflux transporter) and an S269P mutation in *hmg1* (a hydroxymethylglutaryl-CoA reductase) were identified in 4 resistant isolates but not in the initial susceptible isolate (Appendix Table 5, Figure 4), suggesting their roles involved in azole resistance (*4*,*9*). Another 2 pan–azole-resistant respiratory isolates were recovered from a patient (no. 12) who had pulmonary aspergillosis and was receiving voriconazole for 4 months. Azole-susceptible and azole-resistant isolates co-existed in this patient, which echoes the international recommendation suggesting testing multiple colonies (≥5) from a single culture (*1*). *Cyp51A* overexpression and an F262 deletion in *hmg1* (*hmg1*$^{F262\_del}$) were identified in these 2 resistant isolates. Although *hmg1*$^{F261\_del}$ was recently reported in azole-resistant *A. fumigatus* from a voriconazole-exposed patient (*4*), whether *cyp51A* overexpression and *hmg1*$^{F262\_del}$ act synergistically to cause resistance warrants further studies.

Finally, reduced colony sizes were observed in all 6 azole-resistant *A. fumigatus* isolates with wild-type *cyp51A* (Appendix Figure 2). Thus, attention should be paid to select colonies of various sizes for susceptibility testing from patients with azole exposure.

Overall, 4 patients harboring azole-resistant *A. fumigatus* with environmental mutations and 2 patients harboring azole-resistant *A. fumigatus* with wild-type *cyp51A* showed development of invasive aspergillosis, and all had aspergillosis-related deaths. High mortality rates for azole-resistant aspergillosis we observed (6/6, 100%) and for those from a previous report (*10*) emphasize the need for a proposed integrated algorithm for management and control of azole-resistant aspergillosis (Appendix Table 6).

In conclusion, we report a health threat that arose from clinical and environmental use of azoles; environmental use contributed at a larger and global scale. These data necessitate diagnostic stewardship in the clinic and antifungal stewardship in the environment.

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Knowledge of Infectious Disease Specialists Regarding Aspergillosis Complicating Influenza, United States

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In an online survey, we found that nearly one fifth of physicians in the United States who responded had seen or heard about a case of invasive pulmonary aspergillosis after severe influenza at their institution. However, <10% routinely used galactomannan testing to test for this fungus in patients with severe influenza.

Invasive pulmonary aspergillosis (IPA) occurs primarily among immunocompromised patients with a history of organ or stem cell transplantation, chemotherapy, or immunosuppressive medications. However, a multicenter retrospective study in the Netherlands and Belgium suggested that patients