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Emergence of voriconazole-resistant isolates of Aspergillus flavus in Korean hospitals: resistance mechanisms and microsatellite typing analysis

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Background: Although uncommon, azole resistance in *Aspergillus flavus* has been sporadically reported in several regions of the world. We investigated the resistance mechanisms of and genetic relatedness among voriconazole-resistant (VR) isolates of *A. flavus* obtained from a Korean nationwide surveillance study.

Material/methods: A total of 45 *A. flavus* isolates, including 12 VR isolates with voriconazole minimal inhibitory concentrations (MICs) of 2–8 μg/mL and 33 voriconazole-susceptible (VS) isolates with voriconazole MICs of ≤ 1 μg/mL, were recovered from clinical specimens (15 ear, 20 respiratory and 10 others) at nine Korean hospitals from March 2012 to May 2013. *CYP51A, CYP51B* and *CYP51C* genes were sequenced, and the expression of eight genes (*CYP51A, CYP51B*, *CYP51C, MDR1, MDR2, MDR4, Aflatrf,* and *Afmfs1*) were quantified using real-time PCR. Genotyping was performed by microsatellite typing using five markers (AFLA3, AFLA7, AFPM3, AFPM4, and AFPM7).

Results: Of the 12 VR isolates, only one showed a missense mutation (T355A) in *CYP51A*, and none showed missense mutations in *CYP51B.* All 12 VR isolates contained one or more missense mutations in *CYP51C*, including S240, D254G and N423D, but these mutations

were also found in 33 VS isolates. Of the eight genes evaluated by real-time PCR, the mean expression levels of *CYP51A, CYP51B*, *MDR1, Aflatrf*, and *Afmfs1* were significantly higher in the VR isolates than in the VS isolates [VR isolates vs. VS isolates: *CYP51A* (1.791 vs. 1.012)*, CYP51B* (1.409 vs. 1.105), *MDR1* (2.321 vs. 0.6223)*, Aflatrf* (1.394 vs. 1.058), and *Afmfs1* (0.8528 vs. 0.6944), respectively; all $P < 0.05$]. Microsatellite typing of 45 isolates yielded 36 genotypes (types 1–36), of which 32 were unique to a single isolate, while the other 4 were shared by 13 isolates. Interestingly, type 1 was exhibited by six VR isolates that shared the same non-synonymous mutations (S240A/D254G/N423D) in *CYP51C,* all of which were obtained from the ear specimens of six patients from four hospitals.

Conclusions: Our study shows that overexpression of the efflux pump or its target genes, rather than mutations in *CYP51*, could be important resistance mechanisms in the VR isolates of *A. flavus* in Korean hospitals. In addition, one-half of the VR *A. flavus* isolates shared the same microsatellite genotype, suggesting the existence of *A. flavus* clonal isolates.