Occurrence and molecular characterization of triazole-resistant *Aspergillus fumigatus* in outdoor and hospital environment in Kuwait

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Introduction

Aspergillus fumigatus, a common airborne fungal pathogen, causes invasive aspergillosis (IA), a life threatening infection in immunocompromised patients. Triazole resistance is an emerging problem in *Aspergillus* spp. which impacts the management of IA. Triazole-resistant *A. fumigatus* strains have also been isolated from the environment from several agriculturally important European and Asian countries and an environmental source of infection with triazole-resistant strains is increasingly being argued. This study describes the isolation and molecular characterization of triazole-resistant *A. fumigatus* strains from outdoor air and hospital environment in Kuwait.

Materials and Methods

1. Cultures of outdoor and indoor air, water and cotton swab samples from different wards/units of a major tertiary care hospital were obtained by exposing malt extract agar (MEA) medium-containing Petri plates. Soil samples from several locations in Kuwait were also cultured and *A. fumigatus* colonies were identified by phenotypic and molecular methods.
2 Initial screening of drug susceptibility testing of *A. fumigatus* isolates to triazoles was carried out for itraconazole by Etest. Isolates with reduced susceptibility to itraconazole (MIC of >2 mg/L) were also tested for itraconazole, posaconazole and voriconazole by broth microdilution method.
3. Resistance mechanisms involving *cyp51A* mutations were probed by mixed-format real-time (MF-rt)-PCR assays. Triazole-resistant isolates were typed by nine-locus microsatellite analysis. in combination with UPGMA clustering.
4. A multiplex allele-specific (MAS)-PCR assay was developed for detection of L98H mutation in *cyp51A* by simultaneously using three primers (AFCYPF, 5'-AGTTCTTCTTTGCGTGCAGA-3'; AFCYPR, 5'-TTCTCAATAAGTGGCACATGA-3'; AFCYP98H, 5'-CCGCATTGACATCCTTGTG-3') in PCR amplification reactions. The MAS-PCR should yield a single fragment of 354 bp from *A. fumigatus* isolates containing wild-type codon 98 (CTC, L98) in *cyp51A* (*cyp51A98*) and two DNA fragments of 354 bp and 189 bp from *A. fumigatus* isolates containing CAC (H98) at *cyp51A98* (L98H mutation).
5. *A. fumigatus* strain CBS 113.26 containing wild-type (L98) *cyp51A98* and *A. fumigatus* strain VPCI1042/09 containing the L98H mutation at *cyp51A98* were used as reference strains for establishing MAS-PCR.
6. The results of MAS-PCR assay were confirmed by direct DNA sequencing of *cyp51A* codon 98 DNA region from selected isolates.

Acknowledgments

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Results

1. A total of 72 (14, 13 and 45 isolates from hospital ward air/floor swab samples, hospital outdoor air and soil samples, respectively) *A. fumigatus* isolates were grown from various locations in Kuwait.
2. Eight of 72 (3 of 14 from hospital ward air/floor swab samples, 1 of 13 from outdoor air and 4 of 45 isolates from soil samples) isolates were resistant to itraconazole (MIC >2 mg/ml) by Etest. All 8 isolates were also resistant to itraconazole, posaconazole and voriconazole by broth microdilution method.
3. MF-rt-PCR assays detected TR_{34} in promoter region and L98H mutation in *cyp51A* of all 8 triazole-resistant *A. fumigatus* isolates. None of the susceptible isolates contained these alterations.
4. Three microsatellite patterns were observed among the resistant isolates with one pattern clustering with Indian clinical and environmental isolates (Fig. 1).
5. MAS-PCR assay accurately detected wild-type (CTC) sequence in all itraconazole-susceptible isolates and presence of CAC (L98H) mutation at *cyp51A98* in all 8 triazole-resistant *A. fumigatus* isolates (Fig. 2) and DNA sequencing data confirmed these results.

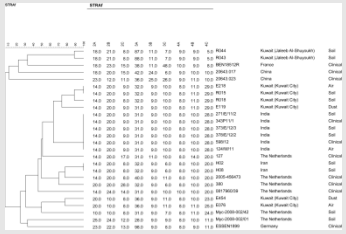

Fig. 1. Genotypic relationship between triazole-resistant *A. fumigatus* isolates from Kuwait with isolates from the Netherlands, Germany, France, India, China and Iran. Dendrogram is based on a categorical analysis of 9 microsatellite markers. The scale bar represents the percentage identity

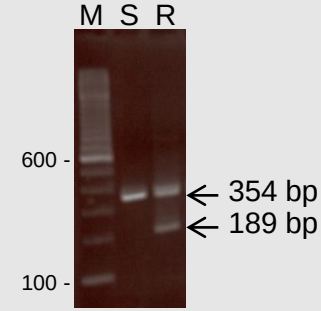

Fig. 2. Agarose gel of MAS-PCR amplicons from triazole-susceptible i(E-424, lane S) and triazole-resistant (E-76, lane R) *A. fumigatus* isolates. Migration position of 354 bp and 189 bp fragments are indicated by arrows.

Conclusions

This study has shown that triazole-resistant *A. fumigatus* isolates are also prevalent in Kuwait and, similar to other studies, the dominant resistance mechanism involved TR_{34}/L98H in *cyp51A*. Presence of triazole-resistant *A. fumigatus* strains in the environment (including health care facilities) suggests that the possibility of susceptible individuals getting infected with such strains exists even in semi-arid, desert countries like Kuwait in the Arabian Peninsula.