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ePoster Viewing
Antifungal drug susceptibility and resistance

Simple, low-cost molecular detection of TR34/L98H mutations in cyp51A gene for rapid detection of triazole-resistant Aspergillus fumigatus isolates

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Objective: Invasive aspergillosis (IA), mainly caused by *Aspergillus fumigatus,* has emerged as a life-threatening infection in immunocompromised patients. Triazoles are the most effective drugs for treatment of IA. Triazole-resistant *A. fumigatus* strains have been isolated from both clinical specimens and environmental sources and the dominant resistance mechanism involves TR_{34}/L98H mutations in *cyp51A*. This study developed low-cost PCR and PCR-RFLP assays targeting the promoter and codon 98 regions for rapid detection of triazole-resistant *A. fumigatus* isolates carrying TR_{34}/L98H mutations for resource-poor settings.

Methods: Reference *A. fumigatus* strains carrying wild-type and mutant sequences in promoter region and codon 98 in *cyp51A* (*cyp51A98*) and 40 itraconazole-susceptible and 35 itraconazole-resistant clinical and environmental isolates were used. Drug susceptibility testing of *A. fumigatus* isolates to triazoles was determined by Etest. PCR assay targeting TR_{34} region was developed to yield varying amplicons that could be easily resolved by 2.5% agarose gels. The L98H mutation was detected by PCR amplification of *cyp51A98* region followed by restriction digestion of amplicons with *Alu*I to yield different RFLP patterns. Results were confirmed by direct DNA sequencing of respective gene fragments from selected isolates.

Results: PCR amplification of promoter region yielded distinct 105 bp and 139 bp amplicons in agarose gels from isolates containing wild-type sequences and tandem-repeats (TR_{34}), respectively. PCR-RFLP assay from all 40 itraconazole-susceptible isolates yielded three (71 bp, 90 bp and 189 bp) fragments indicating wild-type sequence at *cyp51A98*. PCR-RFLP assay from 32 itraconazole-resistant isolates yielded two (71 bp and 279 bp) fragments indicating L98H mutation while 3 isolates yielded wild-type pattern at *cyp51A98*. The latter 3 isolates contained a mutation either at codon 54 or codon 220. All 32 itraconazole-resistant isolates with L98H mutation at *cyp51A98* yielded 139 bp amplicons indicating the presence of TR_{34} while the remaining 3 itraconazole-resistant and all 40 itraconazole-susceptible isolates yielded 105 bp amplicons indicating wild-type sequence in the promoter region. The results were confirmed by direct DNA sequencing of respective gene fragments from selected isolates.

Conclusions: Simple, low-cost methods for molecular detection of TR_{34}/L98H mutations in *cyp51A* gene have been developed for rapid identification of triazole-resistant *A. fumigatus* isolates in resource-poor settings. The methods will help in determining the prevalence of TR_{34}/L98H mutations in *cyp51A* gene in clinical and environmental *A. fumigatus* isolates and may also help in proper management of patients with IA.

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