Simple and rapid detection of TR34/L98H mutations in the *Cyp51A* gene of triazole-resistant *Aspergillus fumigatus* by tetra-primer ARMS-PCR technique

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Introduction: Single nucleotide polymorphism (SNP) detection has been used extensively for genetic association studies. Alteration of the drug target (*Cyp51A*) is the principal mechanism of triazole resistance among *Aspergillus fumigatus* isolates. The most frequently characterized (hotspot) mutation in *Cyp51A* gene is at codons L98 accompanied by a tandem repeat of 34 base pairs in the 5´upstream region of cyp51A (TR34/L98H). Rapid identification of triazole-resistant point mutations is important for management of aspergillosis. Amplification-Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) is an inexpensive, reliable and rapid way to investigation and identification of point mutations. In this study we evaluated detection of TR34/L98H mutations in the *Cyp51A* gene of triazole-resistant *A. fumigatus* by tetra-primer ARMS-PCR technique.

Materials and Methods: Reference *A. fumigatus* strains (10042001/02 and 10042003/04) carrying wild-type and mutant (TR34/L98H) were used for the establishment of ARMS-PCR assays. Optimization of ARMS-PCR was carried out in a step by step manner. In this technique, four primers in one reaction were done for amplification of indicative amplicons in wild-type and triazole-resistant *A. fumigatus* carrying TR34/L98H mutations. The assays were evaluated using 5 susceptible and 10 triazole resistant isolates.

Results: ARMS-PCR assay from reference triazole-resistant *A. fumigatus* isolate containing TR34/ L98H mutations at *cyp51A* yielded 942 bp & 212 bp DNA fragments. PCR amplification from reference *A. fumigatus* isolates containing wild-type sequence yielded 904 bp & 741 bp DNA fragments. The DNA sequencing data confirmed the results of ARMS-PCR assays for all the isolates analyzed in this study. None of the *A. fumigatus* isolates lacking TR34/L98H mutations yielded false-positive results by ARMS-PCR assays.

Conclusion: ARMS-PCR assays developed in this study is a fast, easy, low cast, and user friendly method that may also help in rapid identification of azole resistant *A. fumigatus* carrying TR34/L98H mutations for proper management of patients with invasive aspergillosis in developing countries.

Key Words: *A. fumigatus*, Triazole-resistant, TR34/L98H, ARMS-PCR