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O0833 Standardisation and application of simple and low cost tetra primer ARMS-PCR for detection of TR34/L98H mutation in triazole resistant *Aspergillus fumigatus*

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Background: Invasive aspergillosis is a life-threatening fungal infection. The emergence of azole resistance became a serious problem worldwide. Mutations in the cyp51 gene, particularly TR34/L98H, are responsible for azole resistance. Amplification-Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) is a simple and economical technique for single nucleotide polymorphisms (SNP) detection. The aim of the present study was to evaluate inexpensive, rapid and simple ARMS-PCR assay by using tetra-primers as a reliable method for rapid detection of TR34/L98H mutation in the *A. fumigatus cyp51A* gene in low-resource settings.

Materials/methods: Reference *A. fumigatus* strains 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 20 susceptible and 16 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.

Conclusions: ARMS-PCR assays 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.