

A high frequency of azole resistance due to G54 mutations in *Aspergillus fumigatus* from bronchoalveolar lavage of chronic respiratory diseases patients in a referral chest hospital in Delhi, India using the AsperGenius® Resistance real-time PCR assay and a new G54/M220 assay

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INTRODUCTION

- Azole therapy for invasive, chronic and allergic diseases of lungs caused by *Aspergillus fumigatus* reduce mortality and morbidity in aspergillosis patients.
- A low burden of azole resistance in aspergillosis could be attributed to limited culture yield of respiratory specimens, leading to uncertainty of true triazole resistance
- The present study evaluated the diagnostic performance of AsperGenius®, a multiplex real-time PCR assay to detect *A. fumigatus* and *CYP51A* gene mutations in BAL specimens as compared to direct microscopy and fungal culture.

MATERIALS AND METHODS

BAL specimens

A total of 160 BAL samples received during 2017-18 in a referral chest hospital in Delhi, India were processed for microscopic examination (10% KOH-Blankophor), fungal culture and serum/BAL galactomannan.

Preliminary Screening of azole resistance

Preliminary Screening of azole resistance using voriconazole (1µg/ml) and itraconazole (4µg/ml) supplemented agar plates were done.

Antifungal Susceptibility Testing (AFST)

AFST was carried out using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method, following the M38-Ed3 guidelines against azoles.

CYP51A gene sequencing

All culture positive *A. fumigatus* were subjected to *CYP51A* gene sequencing.

RESULTS

- 83% of BAL specimens were positive for *A. fumigatus* by AsperGenius® assay, out of which 33 (25%) had mutations associated with resistance (table 1)
- In contrast only 23% of BAL specimens yielded *A. fumigatus* in culture and low positivity (10%) on direct microscopic examination was observed.
- Patient details of BAL specimens positive for resistance associated mutations are given Table 2.
- Of 33 BAL specimens that were positive for resistance associated mutations, 7 specimens showed WT genotype by gene sequencing of isolate cultured from single colony of *A. fumigatus* (Table. 3).

DISCUSSION

- A high proportion (83%) of BAL samples harboured *A. fumigatus* which is linked to high burden of COPD, ABPA, CPA and post tuberculosis patients analysed.
- A higher sensitivity of AsperGenius® Resistance multiplex real-time PCR assay (24%) to detect azole resistance as compared to culture (5%).
- G54/M220 multiplex real-time PCR, newly developed assay can detect G54 and M220 and differentiate from WT mutations in *CYP51A*.
- A high burden of G54 mutations is attributed to the patient population with CPA and ABPA who had previously received itraconazole.
- Mixed infections with azole-susceptible and azole-resistant *A. fumigatus* may occur as well but will only be detected if phenotypic testing of multiple colonies.

Conclusions

- We report for the first time direct detection of G54 and M220 mutations using the new AsperGenius® G54/M220 real-time PCR assay in BAL specimens of patients with chronic respiratory diseases in India.
- Assay is highly promising rapid detection method in the clinical microbiology laboratory for direct detection of 4 important *CYP51A* mutations responsible for azole resistance in India.
- The assay can detect mixed infections when conventional fungal cultures are negative.

Table 1. Distribution of genotypes detected by AsperGenius® in BAL specimens.

Genotype	BAL (n=160)
WT	100
G54	22
Y121F/T289A	5
TR34/L98H	4
M220	1
TR34/L98H + G54	1

Figure 1. Melting curve analysis showing double peak due to mixed infection of WT type and G54 mutant.

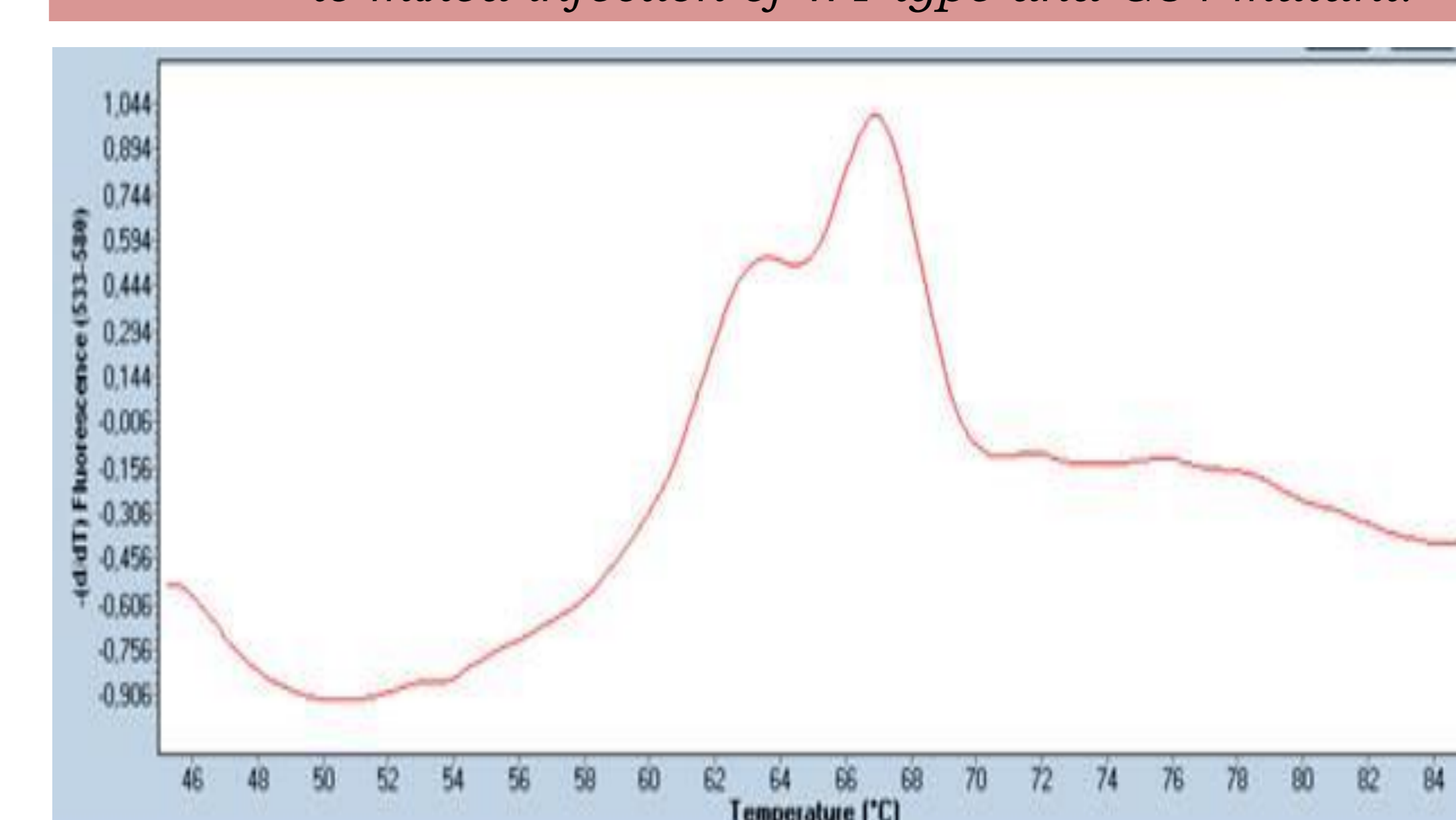


Table 2. Patient details of BAL specimens positive for resistance associated mutations.

Patient diagnosis	No. of cases	Mutation detected by AsperGenius® assay
ABPA (allergic bronchopulmonary aspergillosis)	15	G54 (n=12), Y121F/T289A (n=2), M220 (n=1)
CPA (chronic pulmonary aspergillosis)	10	G54 (n=8), Y121F/T289A (n=1), TR34/L98H + G54 (n=1)
Invasive pulmonary aspergillosis*	5	TR34/L98H (n=3), Y121F/T289A (n=1) G54 (n=1)
COPD (Chronic obstructive pulmonary disease)	3	G54 (n=1), TR34/L98H (n=1), Y121F/T289A (n=1)

Table 3. *In vitro* azole susceptibility and amino acid substitutions in *CYP51A* of *A. fumigatus* detected by AsperGenius® assay and *cyp51A* gene sequencing.

S.No.	VPCI ID	MIC (µg/ml)				Genotype detected by AsperGenius®	Genotype detected by <i>CYP51A</i> gene sequencing
		VRC	ITC	ISA	POS		
1	VPCI_1/17	0.06	0.5	0.06	0.25	WT	WT
2	VPCI_2/17	0.125	0.5	0.25	0.125	WT	WT
3	VPCI_3/17	0.125	0.25	0.25	0.06	WT + G54	WT
4	VPCI_4/17	0.125	0.25	0.06	0.125	WT	WT
5	VPCI_5/17	0.125	0.25	0.125	0.03	WT	WT
6	VPCI_6/17	0.125	0.5	0.125	0.125	WT	WT
7	VPCI_7/17	0.125	0.25	0.25	0.06	WT	WT
8	VPCI_8/17	0.06	4	0.06	1	WT + G54	G54
9	VPCI_9/17	2	>16	2	0.5	TR34 + T289A + WT	TR34
10	VPCI_10/17	0.06	0.5	0.06	0.25	WT	WT
11	VPCI_11/17	0.125	0.5	0.25	0.125	WT	WT
12	VPCI_12/17	0.125	0.25	0.25	0.06	WT	WT
13	VPCI_13/17	0.125	0.5	0.06	0.125	WT	WT
14	VPCI_14/17	0.06	0.25	0.125	0.06	WT	WT
15	VPCI_15/17	0.125	0.5	0.125	0.125	WT + G54	WT
16	VPCI_16/17	0.125	0.25	0.25	0.03	WT	WT
17	VPCI_17/17	0.06	0.5	0.06	0.25	WT	WT
18	VPCI_18/18	0.125	0.5	0.25	0.125	WT + G54	WT
19	VPCI_19/18	0.125	0.25	0.06	0.06	WT	WT
20	VPCI_20/18	0.125	0.25	0.06	0.06	WT + G54	WT
21	VPCI_21/18	0.06	0.5	0.125	0.03	WT	WT
22	VPCI_22/18	0.06	0.5	0.125	0.125	WT	WT
23	VPCI_23/18	0.125	0.25	0.25	0.06	WT + G54	WT
24	VPCI_24/18	0.06	0.5	0.25	0.125	WT	WT
25	VPCI_25/18	0.125	0.5	0.25	0.125	WT	WT
26	VPCI_26/18	0.06	0.25	0.25	0.06	WT + G54	WT
27	VPCI_27/18	0.125	0.5	0.06	0.06	WT + G54	WT
28	VPCI_28/18	0.125	0.25	0.125	0.03	WT + G54	WT
29	VPCI_29/18	0.06	0.5	0.06	0.06	WT + G54	WT
30	VPCI_30/18	0.125	0.25	0.25	0.06	WT	WT
31	VPCI_31/18	0.06	0.5	0.06	0.25	WT	WT
32	VPCI_32/18	0.125	0.5	0.25	0.25	WT	WT
33	VPCI_33/18	0.06	0.5	0.25	0.06	WT	WT
34	VPCI_34/18	0.125	0.25	0.06	0.125	WT	WT
35	VPCI_35/18	0.125	0.25	0.125	0.06	WT	WT
36	VPCI_36/18	0.06	0.5	0.25	0.125	WT	WT
37	VPCI_37/18	0.06	0.5	0.25	0.03	WT	WT