Pyrosequencing of *Aspergillus fumigatus* *cyp51A* reveals triazole resistance when susceptibility testing cannot

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Background

- Acquired polymorphisms in the **lanosterol-14 α -demethylase, *cyp51A***, are responsible for triazole resistance in *Aspergillus fumigatus* isolates.
- Chronic pulmonary aspergillosis (CPA)** and **allergic bronchopulmonary aspergillosis (ABPA)** patients are the main users of the **National Aspergillosis Centre (NAC)**. Many patients produce respiratory samples that fail to culture, preventing susceptibility testing¹.
- Pyrosequencing with the Advanced QIAGEN Q24 system has demonstrated a range of polymorphisms in the *cyp51A* gene from ***Aspergillus* species qPCR positive, high volume culture negative (HVC)** samples (Table 1).
- This method, validated by Sanger sequencing, provides important susceptibility data from a wide range of resistance-associated polymorphisms, many of which are not detected by commercial kits.
- Antifungal drug treatment guided by this screening method is being evaluated to determine whether patient outcome is improved, morbidity is decreased and drug costs are reduced. The case studies below show some preliminary evidence of this.

Results Summary

- Of **204** samples from **113** patients:

Table 1		%
No polymorphisms	65	31.8%
No polymorphisms, some failed regions	41	20.1%
TR34/L98H	9	4.4%
TR34/L98H and G54R	1	0.5%
L98H only	18	8.8%
TR34 only	1	0.5%
G54E/R/V/W	20	9.8%
Y121F and/or T289A	14	6.9%
M220I/K	3	1.5%
M220V and G54R	1	0.5%
M220K and L98H	1	0.5%
Failed	30	14.7%

Case Studies

Case Study 1

64 year old male, CPA, underlying COPD, initial treatment itraconazole. In this case, pyrosequencing suggests change in antifungal drug required.

HVC
No Growth

High volume culture of sputum consistently **negative**. Patient is deteriorating.

HVC
Aspergillus
MIC and Sanger Sequencing

Itraconazole **MIC >8**. Sanger sequencing revealed **wild-type** *cyp51A* profile.

Drug Level
ITRA: toxic

Serum itraconazole drug at toxic levels. Treatment **changed** to **posaconazole**.

HVC
No Growth

High volume culture: no growth, susceptibility testing not possible.

Pyrosequencing of sputum extract: **G54R/M220V** demonstrates resistance to itraconazole and posaconazole.

Case Study 2

41 year old male, *Aspergillus* sensitisation and Hyper IgE syndrome. Initially PCR-positive, on itraconazole. Pyrosequencing used to infer resistance when other methods failed.

HVC
No Growth

Susceptibility testing not possible - lack of growth. ***Aspergillus* qPCR**, sputum sample: **weak positive**.

Drug Level
ITRA: toxic

Drug levels rise and remain consistently high.

HVC
No Growth

No susceptibility testing possible. ***Aspergillus* qPCR**: rise in copies.

HVC
Aspergillus

Pyrosequencing reveals **TR34/L98H** genotype indicating pan-azole resistance.

Four follow-up HVC are positive; susceptibilities support pyrosequence findings.

Case Study 3

71 year old male, early onset CPA. Patient unwell, serum drug levels rising. Pyrosequencing and susceptibility testing together confirm triazole resistance.

HVC
Aspergillus
MIC and Sanger sequencing

Itraconazole and posaconazole **MICs >8**. Sequencing revealed **G54W** *cyp51A* profile.

HVC
Aspergillus

Susceptibilities improve for both drugs.

Drug Levels
VORI: therapeutic

Patient is treated with voriconazole. Over time, serum **drug levels rise**.

Drug Levels
VORI: therapeutic

Pyrosequencing: **L98H/M220K** indicates variable resistance to voriconazole. **No growth** from HVC.

Treatment unchanged, with drug levels continuing to rise. Informed treatment change is warranted.