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Rapid detection of azole resistance with pyrosequencing

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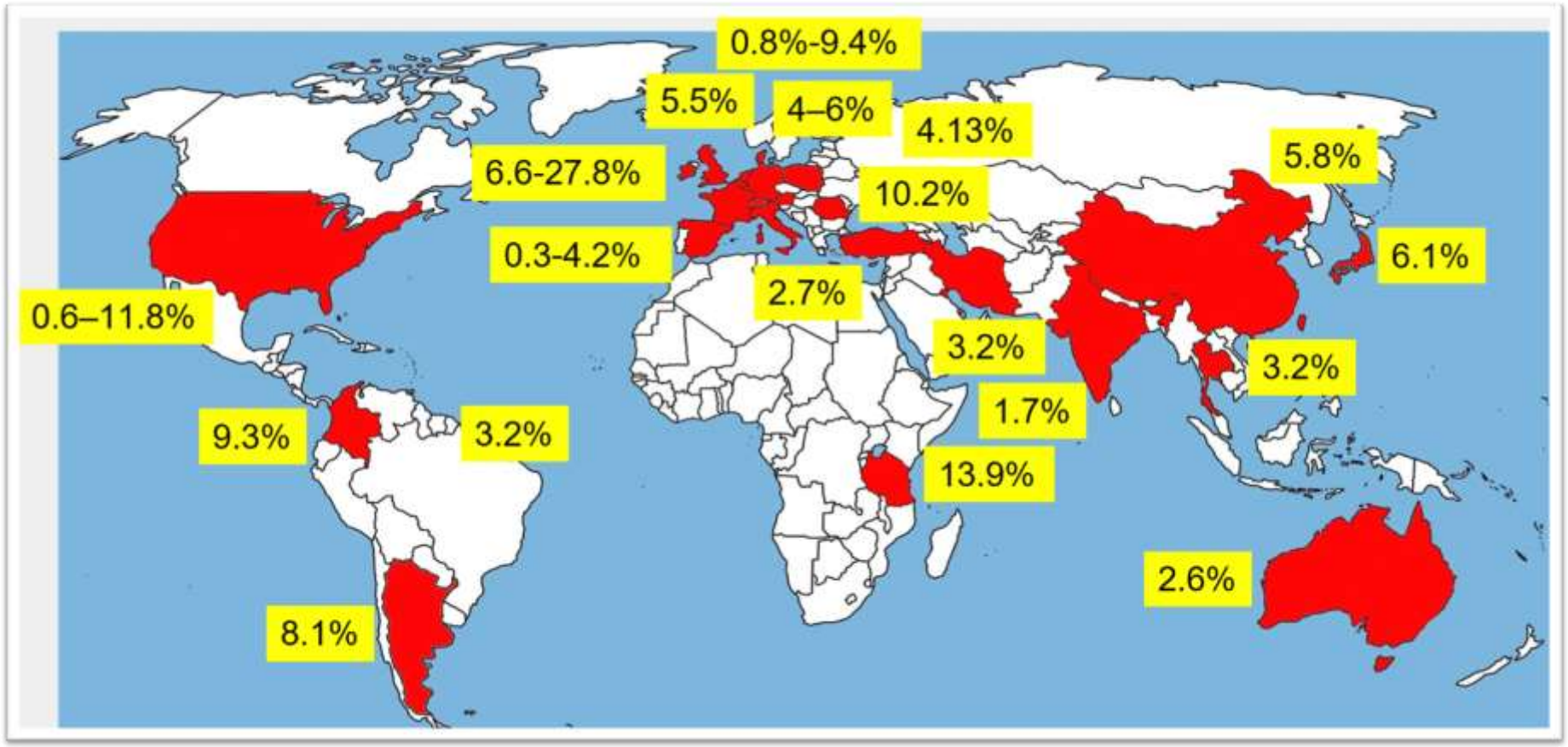
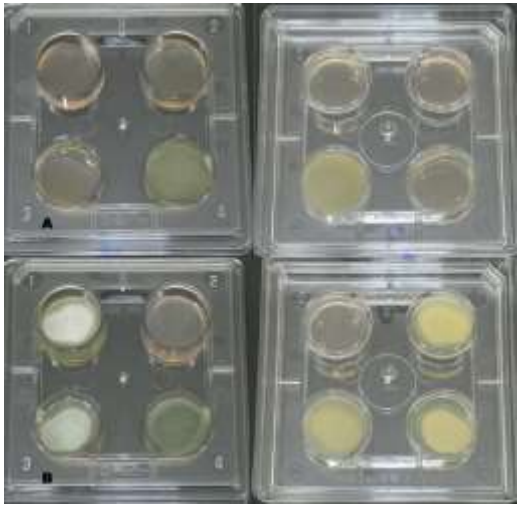
Global *Aspergillus fumigatus* triazole resistance



Adapted from Sharpe AR *et al. Med Mycol* 2018; 56: 83–92, Romero M *et al. J Fungi (Basel)* 2019; 5: 41, and van der Linden JWM *et al. Emerg Infect Dis* 2015; 21: 1041–1044

Current diagnostic methods: Susceptibility testing



- EUCAST
- CLSI
- ETEST (BioMerieux)
- VIPcheck (MediaProducts, the Netherlands)
- High volume culture (HVC) combined with EUCAST/CLSI

pulmonary aspergillosis were not included. The positivity rate of conventional culture was 15.7% (36/229, 95% CI 11.6%–21.0%) and that of HVC was 54.2% (124/229, 95% CI 47.7%–60.5%) ($p < 0.001$). The higher positivity rate of HVC was demonstrated regardless of administration of antifungal treatment. Quantitive real-time PCR had an overall positivity rate of 49.2% (65/132, 95% CI 40.9%–57.7%), comparable to that of HVC.

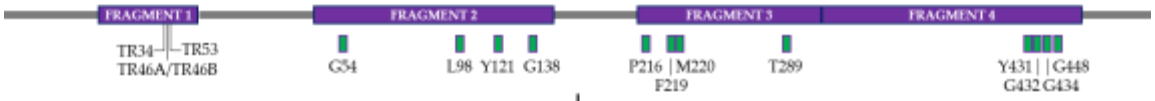
VIPcheck plates: Sensitivity 54–98%, specificity 93–100%

CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; ETEST, EUCAST, European Committee on Antimicrobial Susceptibility Testing; PCR, polymerase chain reaction.

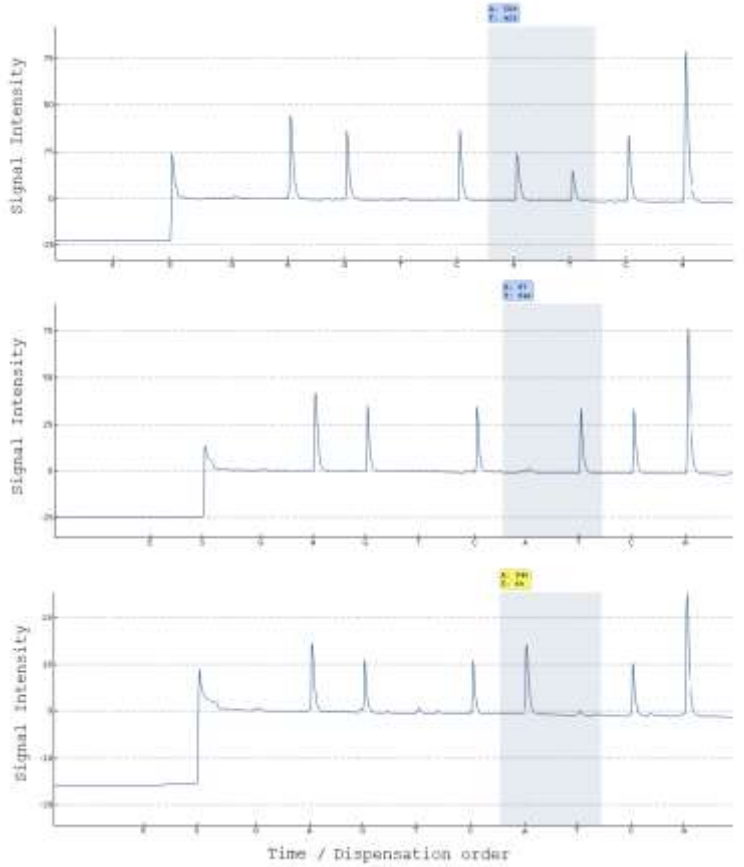
Buil JB *et al. Antimicrob Agents Chemother* 2017; 61: e01250–17;
Lestrade PPA *et al. Clin Microbiol Infect* 2019; 25: 799–806;Vergidis P *et al. Clin Microbiol Infect* 2020; 26: 935–940.

Pyrosequencing

1. DNA extraction of clinical sample (5-6h)
2. Check for 1000 18S copies by *Aspergillus* spp. RT-PCR (1-2h)
3. PCR amplification of four fragments per sample (3h)



4. ssDNA preparation (1h)
5. Pyrosequencing with 7 primers per sample (0.25-1.5h)
6. Analysis of sequencing results (0.25-0.5h)



(ss)DNA, single strand deoxyribonucleic acid; (RT-)PCR, (real time-) polymerase chain reaction.

van der Torre MH *et al. J Fungi (Basel)* 2020; 6: 12;
Novak Frazer L et al. J Antimicrob Chemother 2020; https://academic.oup.com/jac/article/75/12/3501/5899263

Comparison with high volume culture and conventional susceptibility testing

- 326 *Aspergillus* qPCR positive respiratory specimens collected prospectively over 27 months
- 71.2% reported with no *A. fumigatus* growth, targeted for pyrosequencing
- Of these, 56.9% (132/232) demonstrated a WT *cyp51A* genotype and 31.5% (73/232) a resistant genotype
- Pyrosequencing identified the environmental TR34/L98H mutation in 18.7% (61/326) of the samples in contrast to 6.4% (21/326) pan-azole resistance detected by culture

Benefits:

- Detection of both WT and resistant genotypes in same samples
- Detection of *A. fumigatus* templates in samples where growth was obscured by other Aspergilli
- Resistance detected by pyrosequencing earlier than by culture in 23.3% of specimens

qPCR, quantitative polymerase chain reaction; WT, wild type.

Novak Frazer L et al. J Antimicrob Chemother 2020; https://academic.oup.com/jac/article/75/12/3501/5899263

Patient benefit

	Pyrosequenced		Non-pyrosequenced	
	Males	Females	Males	Females
n	32	18	32	18
CPA	25	11	27	12
CFPA	0	1	0	0
ABPA	4	4	5	4
ABPA/CPA	3	2	0	2

Comparison of the distribution and diagnoses of 50 patients whose samples were monitored for resistance by pyrosequencing and matched with 50 patients (for age, sex and diagnosis) whose samples were not sequenced.

CPA, chronic pulmonary aspergillosis; CFPA, chronic fibrosing pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis

Increased resistance detection, lower mortality

- resistance detected by pyrosequencing in 16% (8/50) of HVC negative samples, 12% (6/50) were pan-azole resistant genotype, which as not detected in matched group of patients
- higher mortality rate in non-pyrosequenced group of patients (7/50 vs 4/50)

	Pyrosequenced		Non-pyrosequenced		p value
QoL scores	Males (n = 28)	Females (n = 15)	Males (n = 30)	Females (n = 9)	(Males, females)
Symptom Score	76.8 ± 16.6	67.3 ± 18.5	68.8 ± 20.9	53.0 ± 22.6	0.1903, 0.2094
Impact Score	57.9 ± 24.1	54.9 ± 21.4	47.3 ± 27.1	34.8 ± 28.1	**0.0004, 0.0107**
Activity Score	78.2 ± 22.4	78.6 ± 20.3	72.4 ± 28.7	59.7 ± 32.4	0.8209, 0.0631
Total Score	67.1 ± 21.6	64.1 ± 18.2	58.1 ± 25.1	44.4 ± 28.1	0.2007, 0.1197
	Males (n = 29)	Females (n = 15)	Males (n = 25)	Females (n = 11)	(Males, females)
MRC scores	3.49 ± 1.13	3.13 ± 0.98	3.30 ± 1.36	2.36 ± 0.96	0.9192, 0.0679

Comparison of SGRQ QoL and MRC scores between patient groups. Significance assessed with an unpaired t test ($p<0.05$).

St. George's Respiratory Questionnaire (SGRQ); Quality of Life (QoL) and MRC dyspnoea scores

Future developments

- Streamline the processing
- Further analysis of the benefits and cost savings of using pyrosequencing for resistance detection
- Feed into antifungal stewardship programmes
- Developments to cover more resistance genes

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Manchester Fungal Infection Group (University of Manchester)