# *Aspergillus fumigatus* with prior azole exposure decreases amphotericin B sensitivity in vitro

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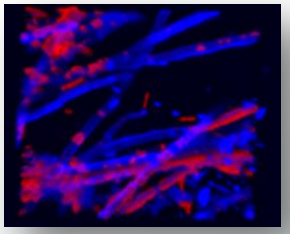
## Introduction

*Aspergillus fumigatus* is a ubiquitous fungal pathogen responsible for aspergillosis in immuno-compromised individuals. Triazoles are the mainstay treatment for majority of fungal infections. Acquired azole resistance in *Aspergillus fumigatus* is increasingly common and is a significant cause of treatment failure. This may result in switching the class of antifungal drug, though the patient may not improve clinically.

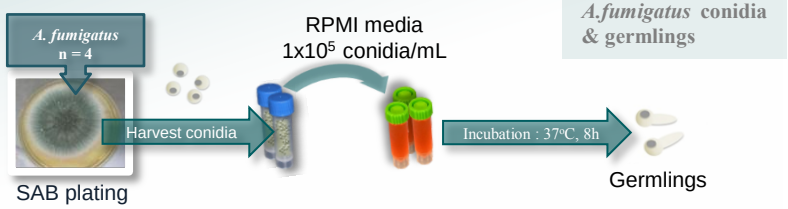
## Aims

The aim of this study was to investigate the impact of prior azole exposure on subsequent amphotericin B (AMB) sensitivity in *A. fumigatus* biofilms. Based on our previous studies on adaptive resistance mechanisms in fungal biofilms, we hypothesised that azole selection pressure activates heat shock protein 90 (Hsp90) and increases extracellular DNA (eDNA) release in *A. fumigatus* biofilms, therefore affecting AMB sensitivity.



*eDNA in A. fumigatus biofilm*
*(Rajendran et al, 2013: Eukaryot Cell)*

## Materials and Methods



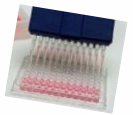

- For time-kill kinetics AMB (1 mg/L) & voriconazole (VRC [1 mg/L]) either alone or in combination (VRC-AMB) were prepared in RPMI and tested against germlings (8 h)

**Susceptibility testing**

- The MIC$_{50}$ of AMB ± DNase (128 mg/L) or geldanamycin (GDA [50 mg/L]) was determined by standard CLSI broth microdilution method
- For azole pre-treatment germlings were treated with VRC at a sub MIC concentration of 0.06 mg/L for 24 h
- Cellular viability determined by the XTT assay

**Imaging**

For scanning electron microscope (SEM), biofilms grown and treated on Thermanox coverslips then biofilms were fixed by aldehydes and viewed under a JEOL JSM-6400 SEM.



XTT assay

## Results & Discussion

### Pharmacodynamics of VRC, AMB alone and in combination against *A. fumigatus* germlings

(i)

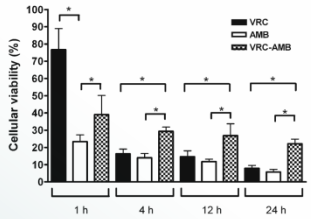



*Figure (i) shows the time-kill kinetics of AMB, VRC or VRC-AMB against germinated conidia (8 h). * p<0.001.*

### Effect of AMB ±DNase/GDA against azole treated *A. fumigatus*

(ii)

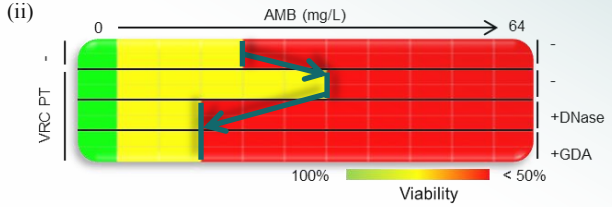



*Figure (ii) shows the MIC$_{50}$ of AMB ± DNase or GDA against either VRC pre-treated (VRC PT) or untreated (-) A. fumigatus.*

(iv) (iii)

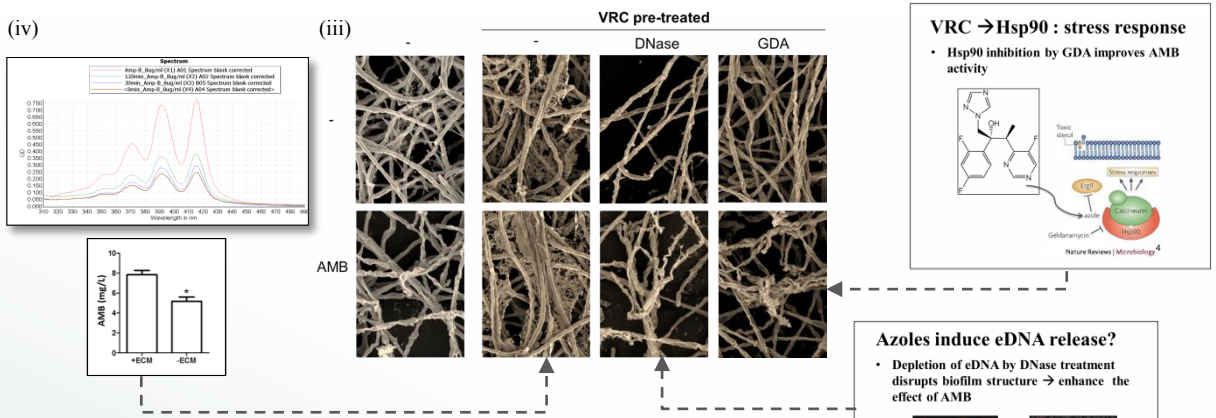



*Figure (iii) SEM images shows the effect of AMB (1 mg/L) against VRC -/PT A.fumigatus biofilm.*
*Figure (iv) AMB absorbance spectra showing the binding of AMB (8 mg/L) with A. fumigatus biofilms (24 h) over the period of 2 h. Bar graph shows the binding of AMB with biofilms in the presence and absence of extracellular matrix (ECM). * p<0.05*

## Summary

These data show that *A. fumigatus* exposure to azole drug induces eDNA release and activates the stress response, which collectively confers AMB resistance *in vitro*. Pharmacological inhibition of these mechanisms may provide novel therapeutic strategies following ineffectual azole therapy.