Introduction

- *Aspergillus* species are ubiquitous fungal saprophytes found in diverse ecological niches worldwide, *A. fumigatus* being the most prevalent.[1]
- *A. fumigatus* is an opportunistic pathogen, a leading cause of invasive aspergillosis (IA) and usually causes infections in immunocompromised hosts resulting in high mortality rates.
- The triazole antifungal drugs (itraconazole, posaconazole and voriconazole amongst others) are the first line drugs used in the treatment of IA however increasing evidence of azole resistant *A. fumigatus* is threatening their effectiveness.
- Triazole resistance can be acquired through two main routes - the prolonged azole therapy or the agricultural route through the use of azole fungicides.
- The latter route is of greater concern; first described in the Netherlands, it is now reported globally and may be the leading mechanism of triazole resistance.[2]
- This emerging phenomenon of triazole resistance in *A. fumigatus* stresses the need for improved new diagnostic strategies i.e. early detection of resistance.
- Currently, anti-fungal susceptibility testing (AFST) is not carried out routinely on all species are ubiquitous fungal saprophytes found in diverse ecological resistant to ≥1 triazole drug. The other 16 strains tested (having previously been shown to be resistant to ≥1 triazole drug by AFST and/or molecular methods in the Microbiology department, SJH) showed growth in one or more of the azole containing wells.

Materials and Methods

- 18 isolates of *A. fumigatus* (sporulating colonies after 2-3 days incubation)
- 1 A293 genome reference strain
- 1 fully susceptible EQA strain
- 16 strains (of clinical and environmental origin) resistant to ≥1 triazole drug

Materials

- VIPcheck™ plates (1 growth control well and 3 wells containing itraconazole 4mg/L, voriconazole 2mg/L and posaconazole 0.5 mg/L)
- Sterile water, swabs, pipette
- Using a wet sterile swab, conidia were collected from a number of colonies (up to 5) of *A. fumigatus* to make a suspension of 0.5-2 McFarland. Each well was inoculated with 25μl of the suspension, incubated at 37°C in air for 48 hours and examined for growth.

Methods

- 2x fully susceptible strains
- Growth in GC well only
- 16 x strains with resistance to ≥1 triazole drug
- Growth visible in ≥1 azole containing wells

Results

<table>
<thead>
<tr>
<th>Strain</th>
<th>Result</th>
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<tbody>
<tr>
<td>2x fully susceptible strains</td>
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Further testing carried out in SJH

- GMIC (Liofilchem™) on RPMI agar to Ampoterinc, Voriconazole, Posaconazole and Itraconazole and/or
- PCR to detect mutations in cyp51A gene (common mutations include TR34/L98H mutations)

Discussion

- A total of 18 strains of *A. fumigatus* were tested using the VIPcheck™ plates.
- The 2 susceptible strains tested showed growth only in the growth control well. The other 16 strains tested (having previously been shown to be resistant to ≥1 of the triazole drugs by AFST and/or molecular methods in the Microbiology department, SJH) showed growth in one or more of the azole containing wells, thus prompting the medical scientist to carry out full AFST.
- VIPcheck™ plates are not intended to give an exact MIC; the preparation of the inoculum has a broad McFarland range of 0.5-2 and only one concentration of each azole drug is included in the plates. Rather, they are useful as a screening method to determine the need for further AFST and/or molecular testing and more importantly for earlier detection of triazole resistance in patients who will require treatment.
- Currently in the microbiology department in SJH, AFST is carried out using gradient MIC strips (Liofilchem™) and results interpreted using EUCAST guidelines.
- VIPcheck™ plates proved to be a reliable screening method for triazole resistance in *A. fumigatus* in SJH and moving forward, will be incorporated in our AFST algorithm for all *A. fumigatus* isolates given the significant cohort of immunocompromised patients in our hospital.

References