Reviewer's report

Title: Molecular Characterization and Antifungal Susceptibility Profile of Aspergillus flavus Isolates Recovered from Clinical Specimens in Kuwait

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Reviewer: Elias Manavathu

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General Comments
In this manuscript Al-Wathiqi, F. et al have investigated the in vitro susceptibility of 92 clinical (collected over a period of 18 years) and 7 environmental A. flavus isolates to 3 different classes of antifungal drugs commonly used for treating fungal infections, including those caused by members of the genus Aspergillus. These investigators initially characterized the isolates using morphological characteristics and subsequently confirmed their identity by molecular biologic techniques such as characterization of the ITS1-5.8S rRNA gene-ITS2 region as well as by partial sequencing of the #tubulin and calmodulin genes. The combined morphological and molecular biologic techniques confirmed that all the isolates these investigators used belonged to A. flavus taxon. Their in vitro susceptibility studies using E-test and disk inhibition assay revealed that both clinical and environmental isolates were highly susceptible to all the antifungal drugs they used except amphotericin B. A low but significant percentage of the clinical isolates were resistant to amphotericin B.

This is a well designed, well performed and well written paper, and the results these authors obtained will be helpful to those studying drug susceptibility of A. flavus isolates. In general, A. flavus isolates have slightly elevated MIC values for amphotericin B compared to those obtained for A. fumigatus isolates, but they are highly susceptible to the triazoles and the echinocandins. It was interesting to note that the drug susceptibility of these A. flavus isolates has not changed significantly over a period of 18 years.

Discretionary Revisions: One of the pitfalls of this study is the use of E-test and zone of inhibition to determine the antifungal activities of the echinocandins, since these authors ignored microcolony formation in their E-test and zone of inhibition. It is unclear how the microcolony formation would affect the interpretation of the results since the echinocandins are fungistatic agents. It is unfortunate that the authors did not perform broth dilution MICs for the echinocandins to compare with their E-test and zone of inhibition data. In addition to this deficiency, the authors should address few minor points (see below) before considering the publication of this manuscript.

Minor Essential Revisions
Line 28: Missing a comma after the word climate. ....geographic regions with
hot and arid climate, including the Middle East.

Line 34: ...... and their susceptibilities to six antifungal agents were determined......

Line 35: The authors use the words Etest and E-test in the manuscript. Which one is accurate? Even if both versions are correct, be consistent in the usage.

Line 65: It should be ..... In 2008, Clinical and Laboratory Standards Institute......

Line 114: What was the concentration of the saline used? 0.9%? Also, ......vortexed for few seconds..... Be specific, 10 S, 15 S, 20 S?

Line 191: Insert the definite article ‘the’ after the word of. ...... Of the 92 clinical isolates tested, 74.2%........

Line 194: ..........at a concentration of #0.256 µg/ml on both test media. At this concentration....

Lines 200-203: Why not include mean ±SD for the MIC values. For example, ..... amphotericin B 1.14 ±SD µg/ml etc.......

Line 315: Spelling mistake. ........to writing of the manuscript. All authors have........

Table 3: Report the geometric mean values with standard deviations. For example 0.842 ± SD etc......

Figures 2 and 3: I am not sure about the significance of Figs. 2 and 3. The greater the antifungal activity of a drug is the larger the zone of inhibition. On the other hand, the better the antifungal activity of a drug is the lower the MIC value. Hence if we plot the E-test MICs (X-axis) vs. zone of inhibition (Y-axis) naturally you would get an inverse relationship.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests