Author’s response to reviews

Title: Use of multiple methods for genotyping of Fusarium solani during an outbreak of contact lens associated fungal keratitis in Singapore

Authors:

Roland Jureen (roland_jureen@alexhosp.com.sg)
Tse H Koh (koh.tse.hsien@sgh.com.sg)
Grace Wang (gptwcy@sgh.com.sg)
Louis YA Chai (chailouis@gmail.com)
Ai L Tan (tan.ai.ling@sgh.com.sg)
Tracy Chai (tracy.chai.l.y@seri.com.sg)
Yong W Wong (yongwee2001@yahoo.com)
Yue Wang (mcbwangy@imcb.a-star.edu.sg)
Paul A Tambyah (mdcpat@nus.edu.sg)
Roger Beuerman (rbeuer@pacific.net.sg)
Donald Tan (snecdt@pacific.net.sg)

Version: 2    Date: 7 April 2008

Author’s response to reviews:

Dear Editor,

Again we thank you and the reviewers for all the time spent going through our manuscript and for excellent comments and suggestions. Below are our answers to the items raised by the reviewers.

Regarding your question on informed consent we have in addition to the previous statement about review board approval added on page 4 line 18: “Written or verbal consent was obtained from all patients or the parents of patients younger than 21 years.”

There are no competing interests and this has been added as suggested by you before Contributions by authors.

We hope that with this response and with the response to the reviewers comments below, the manuscript now will be acceptable for publication.

Reviewer one, David Fredricks

Discretionary 1 and 2.

As noted by the reviewer we did not go into details on perspectives on time and expenses and did not put in a flowchart on the principles behind the methods. The reason for this is mainly to keep the space required as low as possible and because the paper focus on the particular issue of typing Fusarium. Also we feel that this topic has been raised by several papers earlier, most notable maybe in:

Olive MD, Bean P. Principles and applications of methods for DNA-based typing

Minor essential 1.

The reproducibility of this particular AFLP scheme was tested and is reported in the manuscript. The other two methods are referred to in the paper; Rep-PCR (reference 11) and ERIC-PCR (reference 5 and 11). In the references the reproducibility of the methods are mentioned. We checked that we got consistent results when repeating a couple of isolates, but we did not carry out a more extensive test of reproducibility in light of these references.

When running the AFLP samples in triplicates we found that the same isolate was identified as at least 95% similar. “Background noise” could thus falsely increase the discriminatory power if we were to put the level of identity at 95%-100%. The 90% cut-off, which is below the threshold for identity in our triplicate runs, would minimize the risk of this. To have a variability of 95% to 100% similarity when typing the same strain with AFLP is not uncommon and the referred paper 17, which was on typing of enterococci regarded strains as epidemic if the AFLP pattern showed at least 90% similarity. Reference 13, which reports on AFLP typing of Fusarium found that the similarity percent of each group oscillated between 87 and 97%. Where to put the cut off for inclusion into a group is not given, but in light of these references and also to stay clear of the 95% similarity we demonstrated as level of identity in our study we chose a cut off for inclusion at 90% similarity.

We thus feel that variability in each approach does not impact the conclusions since they have been dealt with. We have however clarified the issue in the revised manuscript by inserting the following text on page 6, line 12: “To minimize the possibility of inclusion of an isolate to a group because of test variability and in line with previous investigations on bacteria [17] and Fusarium [13], isolates were assigned to the same AFLP group if the AFLP pattern was at least 90% similar.

This sentence replaces the previous sentence: In line with previous investigations on bacteria [17] and Fusarium [13], isolates were assigned to the same AFLP group if the AFLP pattern was at least 90% similar.

Reviewer two, Patricio Godoy

Minor essential revisions
Page 1, line 11, has been changed according to the reviewers comment
Page 4, line 21 has been changed according to the reviewers comment (now line 23)
Page 14 and 15 has been changed according to the reviewers comment

Reviewer three, Kerry O'Donnell
1,1 Agree, this has now been changed

2.2 All outbreak isolates that were available for the study were members of
FSSC. This has been clarified in the text below.

3.10-11 Agree with the reviewer. This was clumsily worded and has now been changed from “Nucleic acid based methods are often used in laboratories to further speciate Fusarium spp” to “Nucleic acid based methods are often used in laboratories to identify Fusarium spp.”

4.23 (now 4.25) F. incarnatum has been changed to F. cf. incarnatum as suggested. The Fusarium oxysporum was not part of the outbreak, and this has now been clarified on page 5, line 1-2.

5.6 (now 5.9) Agree, this has now been changed.

6.1-11 (now 6.5 to 6.17) The analysis was not band based but curve based. Curve based analysis of AFLP patterns are often used and has been published previously by the first author (Jureen et. al. J Clin Microbiol. 2003 Jun;41(6):2330-6.) and several other groups. Thus the total signal from the Gene scan software was exported into the BioNumerics software and data between 50bp and 500bp were subjected to analysis using the Pearson coefficient which is usually the standard coefficient used for curve based analysis (as opposed to Dice or Jaccard which often are used for band based analysis).

6.16 The Mg2+ concentration was 2mM. This has now been added in the text on page 6, line 25.

11.19-24. (now 12.1- 12.7) Agree. The result has been added as a separate column into Figure 1, so the reader can see exactly how each isolate was grouped with our methods. We have added in the figure text that these results are from reference 3. We have added some lines explaining the difference between the MLST and AFLP (12.3-12.7). We have now included discussion on all ten isolates that were typed in reference three (previously we only discussed the ones that were grouped together in groups containing two or more isolates which was not clear).

12.15 We have in the text above (12.3-12.7) addressed this issue. We also discuss reference 3 and 7 in the discussion. The current line in the conclusion “There was a high degree of diversity among the Fusarium strains included in our study, consistent with earlier reports that this international outbreak was associated with improper contact lens use…” is in reference to these two papers (reference 3 and 7).

Fig1. We have added information explaining location. The “missing” information for Rep-PCR and ERIC-PCR is because the isolates were not grouped with other isolates and would thus constitute a group with just one isolate. All isolates were analyzed except for the few isolates denoted N/A for Rep-PCR in the figure. We have added a line explaining that only groups with two or more isolates are given. As the reviewer requested we have added the 10 isolates where MLST results are available from reference 3. It is common practice that published isolates are made available to fellow researchers. We have in the text on page 5,
line 10-11 added where the isolates are housed should we receive such a request.