Reviewer’s report

Title: Susceptibility Testing of Leishmania spp. against Amphotericin B and Fluconazole using the Sensititre™ YeastOne™ YO9 Platform

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Reviewer: Oghumu Steve

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The research article "Susceptibility Testing of Leishmania spp. Using the Sensititre™ YeastOne YO9 Platform" submitted by Boggild et al. proposes the repurposing of the Sensititre™ YeastOne YO9 drug susceptibility testing platform, currently used in medical practice to determine effective antifungal drug dose ranges against fungal infection, to be used in determination of drug susceptibility for various clinically relevant and standard ATCC strains of Leishmania spp. Using Leishmania spp. promastigotes in media shown not to effect the susceptibility system, Boggild et al. exposed the parasites to a wide range of concentration of two antifungal medications known to have leishmanicidal effect, Amphotericin B (0.12 - 8.0 µg/mL) and Fluconazole (0.12 - 256 µg/mL). After inoculation and a 96 hour drug incubation period, microscopic analysis and AlamarBlue analysis were performed to determine minimum inhibitory concentrations and which strains of Leishmania display resistance to the tested concentrations. Ultimately, Boggild et al. determined that the Sensititre™ YeastOne YO9 system shows promise in being repurposed for its drug susceptibility determination utility against Leishmania, having found with the system that Fluconazole saw no susceptibility of Leishmania spp. at any concentration up to 256 µg/mL, while Amphotericin B showed Leishmania susceptibility between 0.25 µg/mL and 0.5 µg/mL for New World strains and susceptibility at 0.12 µg/mL in Old World strains.

There are some major issues that will need to be addressed.

Since the objective of the paper was to demonstrate the utility of the Sensititre system to determine MICs for leishmania species (a new indication), a more thorough description of the methodological approach and analysis should be provided. For example, how was the colorimetric assay conducted? Alamar blue readings are typically measured by absorbance at 570nm or by fluorescence measurements. Data supporting the correlation between these data and microscopic analysis should be provided.
Were the other drugs in the Sensititre system also measured? There are 9 antimicrobials present in Sensititre. If this was a custom plate, were other relevant antileishmanial drugs performed? Why wasn't this done? Justification should be provided on the narrow scope of drugs used for this study. Further, microscopic analysis and alamar blue assays are traditional assays used for proliferation and cytotoxicity studies. Therefore, a compelling rationale on the advantages of the Sensititre plate was used should be provided.

The figures do not appear to be very informative. What is the point of showing pictures of plates since color changes are very difficult to determine with the naked eye? Besides we do not know what is in each well of the plates shown. Graphs of absorbance readings and correlations with microscopic data should be provided.

What is the point of figure 2? Are there statistical differences between the groups shown. If so where are the differences? The authors indicate that the Fishers exact test was used. What were the results of the tests? Any p values?

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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