Title: Biofilm formation of Clostridium difficile and susceptibility to Manuka Honey

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Author's response to reviews: see over
REVIEWER 1
The manuscript by Eric N Hammond, Eric S Donkor and Charles C Brown “Biofilm formation of Clostridium difficile and susceptibility to Manuka Honey” is addressed to the topical problem of antimicrobial properties of Manuka honey. The methods are appropriate and well described, the discussion and conclusions are well balanced and adequately supported by the data. This work shows scientific novelty. The manuscript is in line with the journals requirements so I suggest accepted with minor revision. Despite the relevance of the research, I suggest making some small corrections in the text.

We thank the reviewer for the positive comments about our manuscript.

I´m not an English speaker so it is difficult to me judge the writing. It is necessary cheek carefully the units. It is need a space between the number and the SI unit. Example: not 1ml but 1 ml.

These corrections have effected in the manuscript (please see methods section in lines 103-170).

There are some mistakes of this in the manuscript. Replace in Figure 1 hrs by hours.

This correction has been effected (please see Figure 1 on page 7)

In 2.1 [Clostridium difficile strains] remove the italic letter from strains.

This correction has been effected (please see section 2.1 on page 4- line 104)

In Material and methods (2.4) – “Different concentrations of Manuka honey (1-50% w/v) were included in the experimental setup and were prepared by dilutions with RCM” It is important the authors specify which are the concentrations tested.

The different concentrations of Manuka honey used have been indicated (please see Section 2.4 on page 5 line 149).

2.5 Statistical analysis
“The experimental data was entered in MS-EXCEL and analyzed” sentence need correction and indicate the version of the program.

This has been done (please see Section 2.5 Statistical analysis on page 6 lines 165-166).

The Student's t-test were applied to differences between biofilms formation as function of the hours and also for different strains? This point must be better explained, presented and discussed in the paper.
The required explanation has been provided under Section 2.5 Statistical analysis on page 6 (please see lines 166-169) and also results section line 174.

The sentence “Several studies have shown that the conventional methods of killing bacteria by using antibiotics and disinfection are often unsuccessful with biofilm forming bacteria [1]” must be modify. If it was several it was need more references. If the intend is use only these reference, several word must be changed or removed

The word “several” has removed (please see page under introduction on page 3 line 57-58). Page 8 – revise sentence: “Our results are also consistent with the findings of Fux et al.”

The sentence has been rephrased to make it more meaningful (please see page 9 line 222-223).

REVIEWER 2

Interesting manuscript, useful and relevant. Well planned experiments and results clearly reported. No major compulsory revisions necessary. No minor essential revisions necessary.

We thank the reviewer for the positive comments about our manuscript.

Discretionary revisions:
The manuscript would benefit from further clarification of the conditions of biofilm growth (temperature and time are described but oxygen conditions are only described for the honey treated portion of the assays). The authors refer to washing the microtiter plates in a microplate reader in the methods section (at the end of page 5), what is the brand?

Oxygen conditions of biofilm growth have been highlighted in Section 2.3 line 122. The culture was done in an anaerobic cabinet.

Washing of the plates in a microplate reader is a mistake and this been corrected in line 157. The microplate reader was used to measure absorbance and the brand of the microplate reader (Dynex plate reader) has been stated in line 162.