Author’s response to reviews

Title: In vitro evaluation of methicillin-resistant and methicillin-sensitive Staphylococcus aureus susceptibility to Saudi honeys

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Author’s response to reviews:

Dr. Liam Messin
Editor-in-Chief
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Dear Dr. Liam Messin

Re: Manuscript reference No. BCAM-D-18-01754

Please find attached a revised version of our manuscript “In vitro evaluation of methicillin-resistant and methicillin-sensitive Staphylococcus aureus susceptibility to Saudi honeys”, which we would like to resubmit for publication as a Research Article in BMC Complementary and Alternative Medicine.
The comments of the reviewers were highly insightful and enabled us to greatly improve the quality of the manuscript. The following pages contain our point-by-point responses to the comments of the reviewers.

Revisions in the text are shown in coloured text. In accordance with the reviewer’s suggestions, we have made changes accordingly. We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make the manuscript suitable for publication in BMC Complementary and Alternative Medicine.

I shall look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Responses to the comments of Reviewer #1

Comments to the Author

1. Authors decided to carry out the agar-well diffusion method for initial testing of antibacterial potential of all honey samples. They used positive control vancomycin. Why the authors decide to use any positive control? For what purpose? Authors do not use phenol as used for Manuka honey testing. Authors simply measured the zone of inhibition with no relation to vancomycin efficacy. In addition, this technique is not
suitable for honey, and authors also describes all limitations of this method in their manuscript. It is not suitable method for complicated matrix as honey is.

Response: We would like to thank the reviewer for these insightful comments. The positive control was used to correlate variations in the agar well diffusion assay [1]. Vancomycin was used in this study as a positive control, which gave a zone of inhibition to monitor day-to-day variations. We preferred vancomycin over phenol because it is a more standardized product and is used for susceptibility testing of MRSA. This is not the first study in which antibiotic discs were used in agar well diffusion assays to monitor day-to-day variations. There are a number of studies in which commercially available antibiotic discs have been used in agar well diffusion assays to ascertain the reproducibility and reliability of the assay [2-4]. We agree with the reviewer comment that the agar well diffusion assay is not a highly sensitive technique to evaluate the antibacterial activity of honey, as we mentioned in our manuscript. However, it is still used in evaluating the antibacterial activity of honey and other natural products, where large numbers of samples are tested, because of its ease, rapidity and low cost [5-7]. Keeping in view the limitations of agar well diffusion, we have also used a microbroth dilution assay, which is more sensitive, and provided quantitative results. However, the disadvantage of the microbroth dilution assay is that it is time consuming and laborious and not suitable when large numbers of samples are used for testing. The purpose of using the agar well diffusion assay in our study was to make a comparison between the results of these two techniques.

2. Authors selected just one Saudi honey for further research and determine the MIC value by microbroth dilution method. The methodology for determination of MIC is confused and not well written or carried out. Authors did not provide information about the final volume in the wells in the plate, what type of plate they used and conditions (stationary vs. shaken). I strongly suggest to test all samples by microdilution method because it is more appropriate than agar well diffusion method.

Response: We would like to thank the reviewer for these insightful comments. As per the reviewer’s suggestion, the methodology for determination of the MIC was explained by adding all the necessary information, incorporated in pages 7-8, lines 154-166. The reviewer’s suggestion regarding testing all honey samples by the microdilution method is invaluable; however, we regret to say that at this stage, it is not feasible for us to test all honey samples with the microdilution method because we have already tested the antibacterial activity of honey samples against both MSSA and MRSA in agar well diffusion assays. In the future, we intend to perform a large antibacterial screening study of honey by the microdilution method. However, the only drawback of microdilution assays is that they are laborious to complete.
3. It is quite weird that all human SA isolates showed same sensitivity to one honey sample. There were no differences in antibacterial activity of each honey against different SA isolates. Authors stated that they used honey dilution (1-20%). What increment was used? 1% or 5%.

Response: We used 1% incremental dilutions for Sumra and Manuka honey and 5% incremental dilutions for artificial or simulated honey, as mentioned in the methodology section (page 8, lines 159-162, 165-166). The same MIC of one honey against different isolates of bacteria of the same species is not a novel finding. This pattern has been shown in several other studies [8, 9]. In one study, all isolates of S. aureus, including both resistant as well as sensitive strains of S. aureus, were inhibited at exactly the same honey concentrations (4% v/v) [10]. Honey is effective regardless of the drug resistance profile of the isolates, with multidrug-resistant strains being just as susceptible to its action as drug-sensitive strains [11].

4. Both figures should be removed because you doubled the data. All data shown in both figures are present in Table 2 and 3. No redundancy is allowed.

Response: Thank you for the suggestion. As per the reviewer’s suggestion, both figures have been removed.

5. English language needs to be checked by English native speaker. There are numbers of typographical errors.

Response: As per the reviewer’s suggestion, the manuscript has been checked by Nature Research Editing Service and corrected accordingly.

Responses to the comments of Reviewer #2

1. The title should include both, S. aureus methicillin-resistant and S. aureus methicillin-sensitive, once both were evaluated. The authors could put in the title S. aureus without any specification, if they would rather that.

Response: Thank you for the reviewer’s suggestion. The title has been modified accordingly.

2. The geographical coordinates of each honey sample should be indicated;
Response: As per the reviewer’s suggestion, the geographical coordinates are indicated in Table 1.

3. The honey producers bee species should be indicated;
Response: As per the reviewer’s suggestion, the name of the bee species has been added (page 6, lines 113-115).

4. The collecting months of each honey sample should be informed;
Response: As per the reviewer’s suggestion, the collection months have been added in Table 1.

5. The discussion about the antibacterial activity of other constituents present in honey samples, except for peroxides, could be improved.
Response: As per the reviewer’s suggestion, the antibacterial activity of other constituents present in honey samples, except for peroxides, was discussed and incorporated on page 11, lines 241-247.

Reviewer comments # 3

1. Abstract: The results section should contain both zone of inhibition and MIC data. It is currently heavily weighted towards the zone of inhibition data, which is arguably not the best way to measure activity. MIC testing is a more reliable way to test for activity.
Response: As per the reviewer’s suggestion, the abstract has been modified accordingly (pages 2-3, lines 41-46).

2. Line 71: Rather than state that prevalence is "quite high", please state actual prevalence values, and compare to other countries.
Response: As per the reviewer’s suggestion, the actual prevalence rate has been mentioned and compared with that in other countries (page 4, lines 72-77).
3. Line 78: Similarly, a number of clinical trials have shown that honey was worse than standard treatment. This topic must be discussed fairly, showing all sides of the story.

Response: As per the reviewer’s suggestion, the findings of clinical trials that reported adverse effects of honey are also included (pages 4-5, lines 90-95).

4. Line 85: What is meant by certified? Are these honeys registered with therapeutic goods authorities?

Response: Yes, the certified honeys are those that have been approved and registered by health regulatory authorities. This information has been incorporated in the text (page 5, lines 98-9).

5. Line 101: Please explain "sterility was checked on blood agar medium". How much honey was tested?

Response: As per the suggestion of the reviewer, the required information has been incorporated into the manuscript (page 6, lines 116-118).

6. Were honeys completely sterile? Most honeys will contain at least a few bacterial endospores and will therefore not be sterile.

Response: The tested honeys did not show any growth on blood agar plates. However, we agree with the reviewer’s opinion that honey may contain different species of lactobacilli or bifidobacteria originating from bee stomach. Lactobacillus and Bifidobacterium, two of the most important genera within the lactic acid bacteria group, are commonly found as commensals and are used as probiotics for humans and animals [12]. However, these bacteria need special media to grow, such as MRS, and require anaerobic conditions. Occasionally, honey may contain spores of Clostridium botulinum and environmental pollutants if the honey was not harvested properly [13, 14]. Therefore, it is recommended that honey be gamma irradiated to make it sterile before its clinical application on wounds [15].

7. Line 123: Which strains were used? Was it all strains?

Response: Yes, all strains were used (page 7, lines 154-5).
8. Line 137: Does this mean that the experiment was performed three times on different
days (ie independent biological repeats), or were three identical wells used on the same
day.

Response: Thank you for this inquiry. The term triplicate has been clarified. All experiments
were performed in triplicate on the same day using three identical wells (page 7, lines 154-156).

9. Line 143: Please clarify what the final concentrations of honey were in the tray. Also how
were honey solutions prepared? It is very difficult to pipette honey so was it weighted out
instead?

Response: As per the reviewer’s suggestion, all the above-mentioned queries are explained in the
methodology section (page 7-8, lines 156-9).

Line 151: As above, clarify what is meant by triplicate.

Response: As per the suggestion of the reviewer, the term triplicate has been clarified (page 7,
lines 151-2).

10. Line 183: Always state the method by which the results were generated. For example,
"exhibited higher antibacterial activity by agar diffusion…"

Response: As per the reviewer’s suggestion, the name of the method has been mentioned (page 2,

11. Line 187: It is not clear why these honeys would have high peroxide activity from the
results described in lines 183-185. These results do not describe catalase, so no
conclusions can be drawn about peroxide activity.

Response: As mentioned in the methods section, two types of diluents were used in the agar well
diffusion assay: sterile distilled water and a catalase solution. The results mentioned in lines 183-
185 are related to the antibacterial activity of honey diluted in sterile distilled water, where
peroxide activity remains intact because catalase was not used in these dilutions. Therefore, the
zone of inhibition represents the total or peroxide activity of honey and thus can be concluded as
such.

12. Line 236: Although the difference in MICs (12% compared to 14%) was statistically
significant, the biological or clinical significance should also be mentioned. Do the
authors think that such a small difference in MIC would translate into a significant difference in a clinical study?

Response: We would like to thank the reviewer for such insightful comments. We think that such a small difference (less than 3%) in the MIC of tested honeys would not make any significant difference in a clinical study because undiluted honey is usually applied to infected wounds and burns. After topical application, if honey is supposedly diluted by wound exudate by more than 50%, it would not affect its efficacy because both honeys have MICs less than 15%. According to previously published studies, it has been shown that differences in MICs of different honeys less than 5% are not significant clinically [16]. Currently, only UMF (unique Manuka factor) honeys or methylglyoxal-containing honeys are registered as therapeutic agents for wound infections. However, there was a small difference (<5%) between the antibacterial activity of UMF honeys and other honeys, as observed by the MIC assay [17]. The significance of these differences in a clinical setting is also unclear; therefore, it is extremely important that the efficacy of UMF and non-UMF honeys be evaluated in randomized controlled clinical trials for the treatment of infected wounds and burns [18]. In addition, bacterial resistance was not induced against either UMF honeys or non-UMF honeys [19]. The question arises as to whether the Manuka grade is a real index of its overall value and whether it is actually or always the best available ‘honey dressing’[20]. As per the reviewer’s suggestion, the clinical significance of the difference in MICs of tested honeys has been mentioned on pages 13-14, lines 285-294.

13. General comment: Include comments on the species of floral sources for the honeys in the discussion.

Response: As per the reviewer’s suggestion, a paragraph has been incorporated regarding floral sources of the honeys (page 10-11, lines 219-227).

14. are these honeys from Apis mellifera bees or another species.

Response: All honey samples were produced by Apis mellifera jementica (the local bees) page 6, line 113-5.

15. Also, some comment could be made on the colours of the honeys - were they all uniformly dark or were some light?

Response: As per the reviewer’s suggestion, honey colours were mentioned in the discussion section (page 10-11, lines 224-227).
16. Table 2 and 3: Change zone sizes to one decimal place only
Response: As per the reviewer’s suggestion, we changed zone size values to one decimal place in Tables 2 and 3.

17. Table 4: Express all numbers with 1 decimal place for all values
Response: As per the reviewer’s suggestion, we expressed all numbers with 1 decimal place for all values in Table 4.

18. Table 4: Italicise E. coli and correct ATCC number to 25922
Response: As per the reviewer’s suggestion, we have modified the terms accordingly in Table 4.

19. Figures 1, 2 and 3 are not required, as the data is shown in Tables.
Response: As per the reviewer’s suggestion, we have removed the figures.

References


