**Reviewer Comments**

**Decreased Methicillin-Resistant Staphylococcus Aureus (MRSA) surface colonization when exposed to medical grade (Manuka) honey alone or in combination with other disinfectants**

***First reviewer’s comments:***

The submitted manuscript entitled “**Decreased Methicillin-Resistant Staphylococcus Aureus (MRSA) Surface Colonization When Exposed to Medical Grade (Manuka) Honey Alone or in Combination with Other Disinfectants**” addressed therapeutic application of honey as standalone or in combination with disinfectant. Authors investigated clinical application of honey against bacterial infection. The manuscript is informative and recommended for publication after minor revision as suggested below:

**Comment** 1: In abstract section, please replace “CFU” with “CFU/mL, “%” with “%v/v”.  These keywords must be as “Manuka; Honey; Porphyrins; MRSA; Disinfectant”.

**Comment 2:** Introduction section should be revised for: (a) please include a short information about the pathogenicity and infections caused by “methicillin-resistant Staphylococcus aureus”. Moreover, the choice of drugs available to control *methicillin-resistant Staphylococcus aureus* caused infections. How these antibiotics are related to cause various side effects? Moreover, the use of honey as an alternative to these established antibiotics as a standalone or in combination.

**Comment 3:** The sentences “Extensive research has been conducted to mitigate the negative health impacts of MRSA, but due to its robust resistance to antibiotics (namely methicillin, which is in part where the bacterial species gets its name), this has been a daunting task, with recent evidence indicating that MRSA is evolving quickly enough to circumvent antibiotics that have recently been given commercial clearance” should be rewritten to make it clear and concise. The sentence “In fact, the U.S. Centers for Disease Control have indicated that by 2050, every 3 seconds someone will die from an antibiotic-resistant bacteria” must be cited using the link of online source with the date of access.

**Comment 4**: The sentence “Manuka honey presents itself as a very strong candidate as a compound to mitigate bacterial growth” must be revised as the honey is not a compound. Please include more information about the honey for its source, its major composition and the prime components responsible to cause antibacterial action such as hydrogen peroxide and methylglyoxal (MGO). I recommend to explain three signature compounds DHA, Leptosperin, and MGO present in the honey and their chemical structure (as figure).

**Comment 5:** The sentences “For example, ginseng and ginseng extract have both been long studied antimicrobial materials that are derived from naturally occurring, organic ingredients (12). Mechanistically, ginseng is able to alter the motility of certain bacteria and viruses, as well as disrupt biofilms that might be relevant to either of the microbe groups (7). Moreover, ginger has proven to be extremely efficacious at disrupting bacterial replication under lab circumstances, and is often used in traditional homeopathic remedies to infection, in a similar vein as ginseng. Ginger has a myriad of medicinal uses, including but not limited to anti-tumor activity through cellular apoptosis, general antimicrobial activity, and inflammation reduction” must be replace with recent advances in the honey and therapeutic applications reported before. Moreover, possible side effect of the honey? (such as allergic reaction, hyperglycemia, and contraindications with food and drugs). Its constituents responsible for tissue regeneration (please refer to the published article doi: 10.2174/1389200218666170911152240).

**Comment 6:** The sentence “Further research is required to holistically understand the full scope of antimicrobial mechanisms at play with respect to Manuka honey, but it is well understood that Methylglyoxal plays a significant role” must be revised in terms of both MGO and H2O2. Both are responsible for antibacterial actions. Nolan et al. reported a systematic review about the honey and MIC against various bacterial pathogen. I recommend to present a major finding as a paragraph of the review (doi: 10.3390/antibiotics9110766).

**Comment 7:** There must be consistency in using any unit such as “hrs” or “hours” or “hour”.

**Comment 8:** In material section, the source of the chemicals must be explained with city, and country name.

**Comment 9:** In the sentence “The green solution was centrifuged” authors could not reveal any time of the process and the model of the instrument (centrifugation and lyophilizer).

**Comment 10:** References should be as per the Journal guideline.

Completed: **2023-01-21 08:14 AM**

Recommendation: **Revisions Required**

***Second reviewer’s comments:***

The authors developed a study aimed to investigate the effectiveness of Medical Grade Manuka honey as a surface disinfectant against methicillin-resistant Staphylococcus aureus (MRSA) in vitro. The study found that a 1% solution of Manuka honey reduced MRSA colonization by 1 log after 24 hours when seeded at a starting concentration of 108 colony forming units of MRSA. The study also found that similar results were achieved when Manuka honey was combined with isopropyl alcohol, a porphyrin solution and citric acid, indicating that Manuka honey could be a suitable, safe, environmentally friendly and effective MRSA disinfectant for everyday household use.

A couple of comments:

1. Although there is still a lot to do to develop an antibacterial based on honey, I would like the authors to include some discussion about the aspects of using such platform in the clinical setting, specifically discussing about biocompatibility and potential immune response triggers.
2. I am missing a more through discussion about why Manuka honey is a good candidate, rather than just saying that because similar candidates have been developed in the past. I would like to see a straight answer to that question: “why honey and nothing else?”
3. There are several aspects of honey as an antimicrobial that need more discussion and references. For instance, honey has a low pH -the low pH of honey (typically around 3.2-4.5) creates an acidic environment that is inhospitable to many bacteria; also, its hydrogen peroxide content -honey naturally contains hydrogen peroxide, an antiseptic that can kill bacteria; and the fact that some types of honey have been found to have additional antibacterial properties that are not due to hydrogen peroxide, but are due to other compounds such as methylglyoxal and leptosperin. Lastly, its osmotic effect should be discussed as well -honey has a high sugar content, which can dehydrate bacteria and inhibit their growth.
4. I would like to see more discussion about a potential targeted application in the antibacterial setting. For instance, honey has been found to have anti-inflammatory properties that can help reduce inflammation and promote healing in infected wounds.
5. In figure 1, include a statistical analysis, such as t-test, that compares all groups to a control. Is there a way to include methicillin as one of the antibacterial agents (known resistance of MRSA to it) as a control?

**For editor only**

The paper's content is indeed interesting but needs some refining, and definitely more discussion in the aspects I commented above.

Completed: **2023-01-22 10:35 PM**

Recommendation: **Revisions Required**

**Authors’ Feedback**

We would like to thank the reviewers for their comments on our manuscript. It has been revised accordingly and brief responses to the reviewers appear below.

**Reviewer A:**

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Response: This has been corrected.

Comment 2: Introduction section should be revised for: (a) please include a short information about the pathogenicity and infections caused by “methicillin-resistant Staphylococcus aureus”. Moreover, the choice of drugs available to control methicillin-resistant Staphylococcus aureus caused infections. How these antibiotics are related to cause various side effects? Moreover, the use of honey as an alternative to these established antibiotics as a standalone or in combination.

Response: This was added to the manuscript.

Comment 3: The sentences “Extensive research has been conducted to mitigate the negative health impacts of MRSA, but due to its robust resistance to antibiotics (namely methicillin, which is in part where the bacterial species gets its name), this has been a daunting task, with recent evidence indicating that MRSA is evolving quickly enough to circumvent antibiotics that have recently been given commercial clearance” should be rewritten to make it clear and concise. The sentence “In fact, the U.S. Centers for Disease Control have indicated that by 2050, every 3 seconds someone will die from an antibiotic-resistant bacteria” must be cited using the link of online source with the date of access.

Response: This was added to the manuscript.

Comment 4: The sentence “Manuka honey presents itself as a very strong candidate as a compound to mitigate bacterial growth” must be revised as the honey is not a compound. Please include more information about the honey for its source, its major composition and the prime components responsible to cause antibacterial action such as hydrogen peroxide and methylglyoxal (MGO). I recommend to explain three signature compounds DHA, Leptosperin, and MGO present in the honey and their chemical structure (as figure).

Response: This was added to the manuscript, although we do not think a figure of the chemical structure of DHA, Leptosperin, or MGO will add anything to the manuscript so we chose to omit it.

Comment 5: The sentences “For example, ginseng and ginseng extract have both been long studied antimicrobial materials that are derived from naturally occurring, organic ingredients (12). Mechanistically, ginseng is able to alter the motility of certain bacteria and viruses, as well as disrupt biofilms that might be relevant to either of the microbe groups (7). Moreover, ginger has proven to be extremely efficacious at disrupting bacterial replication under lab circumstances, and is often used in traditional homeopathic remedies to infection, in a similar vein as ginseng. Ginger has a myriad of medicinal uses, including but not limited to anti-tumor activity through cellular apoptosis, general antimicrobial activity, and inflammation reduction” must be replace with recent advances in the honey and therapeutic applications reported before. Moreover, possible side effect of the honey ?(such as allergic reaction, hyperglycemia, and contraindications with food and drugs). Its constituents responsible for tissue regeneration (please refer to the published article doi: 10.2174/1389200218666170911152240).

Response: This was added to the revised manuscript.

Comment 6: The sentence “Further research is required to holistically understand the full scope of antimicrobial mechanisms at play with respect to Manuka honey, but it is well understood that Methylglyoxal plays a significant role” must be revised in terms of both MGO and H2O2. Both are responsible for antibacterial actions. Nolan et al. reported a systematic review about the honey and MIC against various bacterial pathogen. I recommend to present a major finding as a paragraph of the review (doi: 10.3390/antibiotics9110766).

Response: This was added to the revised manuscript.

Comment 7: There must be consistency in using any unit such as “hrs” or “hours” or “hour”.

Response: This was corrected throughout.

Comment 8: In material section, the source of the chemicals must be explained with city, and country name.

Response: This was corrected.

Comment 9: In the sentence “The green solution was centrifuged” authors could not revealed any time of the process and the model of the instrument (centrifugation and lyophilizer).

Response: This was corrected and was referenced to a prior study.

Comment 10: References should be as per the Journal guideline.

Response: This was corrected.

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