Reviewer's report

Title: Tomatidine and analog FC04-100 possess bactericidal activities against Listeria, Bacillus and Staphylococcus spp

Version: 0 Date: 01 May 2017

Reviewer: Gian Maria Rossolini

Reviewer's report:

This submission reports on an extended characterization of the antibacterial activity of Tomatidine, a natural product from plants, against normal phenotypes and SCV of some Gram-positive species belonging to the Firmicutes, and on characterization of FC04-100, a modified tomatidine derivative, along the same line.

Activity against SCV of some species (e.g. staphylococci) can be an interesting feature given their role in some persistent infections. The relevance of activity against other species (e.g. Bacillus) remains less clear, but those results support a more general activity of these compounds and therefore could be worthy of reporting.

The paper is overall well written, but some issues should be considered for manuscript improvement, as detailed below.

1. Title: the reason for mentioning Listeria as outstanding species is unclear; it was one of the several species tested, but most additional experiments were carried out with staphylococci.

2. L. 37-50: this part of the introduction could be condensed by reducing the taxonomic details.

3. L. 54: here you might wish to mention the importance of MRSA also in veterinary settings (also considering the focus of the paper).

4. L. 149 and ref. 24: ref 24 does not seem appropriate; the correct CLSI reference for broth microdilution methodology is M07-A10 (2015).

5. L. 222-224: the dosages and mode of administration of tomatidine and FC04-100 should be better described here.

7. Results, text and Table 1: the lowest MIC values should be determined and reported (<0.03 is not precise).

8. Results, MIC values (Tables 1 and 2): presentation of the MIC data of tomatidine and FC against various strains, alone and in combination with GEN, could be condensed and streamlined; these data could probably be easier to follow if presented in a single Table and discussed in a single subsection of results.

9. Results, kill kinetics: as mentioned for MIC testing, even in this case an effort should be done at presenting together data for bactericidal activity of tomatidine and FC, rather than in separate sections, to facilitate comparative analysis and streamline the text.

10. Results: the significance of data from the pulmonary infection model appears to be quite preliminary; these data should probably deserve to be expanded (also in combination with antibiotics) and reported in a future publication.

11. Authors contributions: what are the Xs?

12. Fig. 7 legend: per gland? Please clarify

**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.

Yes
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

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments
which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal