Author's response to reviews

Title: Virulence-related traits of epidemic Acinetobacter baumannii strains belonging to the international clonal lineages I-III and to the emerging genotypes ST25 and ST78

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Author's response to reviews:

Tse Hsien Koh
Associate Editor
BMC Infectious Diseases

RE: revised version of MS: 9069575259440913 “Virulence-related traits of epidemic Acinetobacter baumannii strains belonging to the international clonal lineages I-III and to the emerging genotypes ST25 and ST78”

Dear Dr. Koh,

Thank you for the detailed review of our manuscript and for the constructive criticisms provided. We have carefully examined all the points raised by the Reviewers and the Editor and modified the manuscript accordingly. A point by point response to all comments raised by the Reviewers and the Editor with the description of the changes is listed below. All the changes have been highlighted in yellow along the text of the revised manuscript.

We believe that the manuscript has been substantially improved upon revision, and we hope that it will meet with your final approval.

Yours sincerely,

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Answers to Editorial Comment

Specific comment
Please clarify whether any form of ethical approval was required for this study and whether you had any access to patient data. Kindly include a statement within the methods section clarifying this.

Answer

As per Editor’s request, we have clarify in the Methods section that “Epidemiological features of strains and number of patients involved in the outbreaks were in accordance to previous publications [4,28-31]. No ethical approval was required for the study because there was no access to patient data.” (lines 99-101 of the revised manuscript).

Answers to reviewer 1

We thank reviewer 1 for the positive evaluation of our study and for the detailed criticisms provided.

Specific comment

1. Throughout the manuscript replace “adhesion and invasion of cells” by “adherence to and invasion of cells”.

Answer

“Adhesion and invasion of cells” was replaced by “adherence to and invasion of cells” throughout the manuscript.

Specific comment

2. Throughout the manuscript replace ‘lethality of Acinetobacter’ by ‘the ability of Acinetobacter to kill’ or by ‘the virulence of Acinetobacter in vivo’.

Answer

“Lethality of Acinetobacter” was replaced by “the virulence of Acinetobacter in vivo” or “the ability of Acinetobacter to kill” throughout the manuscript.

Specific comment

3. Page 2, line 31-32. The ability to form biofilm was significantly higher for…compared to other PFGE types.

Answer

In the revised manuscript (lines 30-31), we now state that “The ability to form biofilm was significantly higher for A. baumannii strains assigned to ST2 (international clone II), ST25 and ST78 compared to other STs.”

Specific comment

4. Page 2, line 35. Replace ‘positively correlated with’ either by ‘a positive correlation exists between adhesion and biofilm formation’ or by ‘adhesion and biofilm formation were associated’.

Answer

“Positively correlated with” was replaced by “a positive correlation exists between adherence and biofilm formation” (lines 34-35 of revised manuscript).
Specific comment
5. Page 2, line 38. What is meant with sustained biofilm growth?
Answer
“Sustained biofilm growth” was replaced by “high biofilm-forming capacity” (line 38 of revised manuscript).

Specific comment
Answer
Comma was removed after infections (line 49 of revised manuscript).

Specific comment
7. Page 3, line 53. Replace ‘strains, initially named..’ by strains, belonging to the initially named...
Answer
The sentence was modified as recommended by the Referee (lines 52-53 of revised manuscript).

Specific comment
8. Page 3, line 63-64. Replace ‘are biofilm-formers’ by ‘were biofilm-formers’
Answer
“are biofilm-formers was replaced by "were biofilm-formers" (line 63 of revised manuscript).

Specific comment
9. Page 4, line 71-72. Replace ‘contribute to persistence’ by ‘contribute to their persistence’.
Answer
“Contribute to their persistence” was added on line 71 of revised manuscript.

Specific comment
10. Page 4, line 90 Replace ‘evaluated in’ by ‘evaluated for’.
Answer
“Evaluated in” was replaced by “evaluated for” (line 90 of revised manuscript).

Specific comment
Answer
“Assay” was replaced by “assess” (line 118 of revised manuscript).

Specific comment
12. Page 5, line 121. Include ‘to assess the induction of biofilm formation in the presence of antibiotics, the bacteria were…’

Answer
The sentence was modified as recommended by the Referee (line 118 of revised manuscript).

Specific comment

13. Page 6, line 128. For specific details on the method, readers can look up the reference, but it should be described in short how pellicle formation is analyzed.

Answer
The procedure for the analysis of air-liquid pellicle was described on lines 126-127 of the revised manuscript.

Specific comment

14. Page 6, line 136. Is each strain stored in a separate box? How are viable counts performed?

Answer
In the Methods section, we now explain that “Strains were distributed into four separate boxes; each box stored up to six strains. Viable cell counts were determined once a week as previously described [23].” (lines 135-137 of revised manuscript).

Specific comment

15. Page 6, line 140. Place reference after ‘as described previously [15], with minor modifications.’

Answer
The position of reference was moved as suggested (line 140 of revised manuscript).

Specific comment

16. Page 6, line 144. Include ‘adherent and invaded bacteria’ as this method cannot distinguish between adherent and invaded bacteria.

Answer
The sentence was modified as recommended by the Referee (line 144 of revised manuscript).

Specific comment

17. Page 7, line 148. Include ‘killing of all extracellular bacteria’. Is killing achieved within 30 minutes for all strains?

Answer
The adjective “extracellular” was included as suggested. The information that “all strains included in the study were killed after a 30 min treatment with 5 mg/L of
colistin” was also provided. (lines 147-148 of revised manuscript).

Specific comment
18. Page 7, line 159. Triplicate means that 30 larvae were infected with each strain?
Answer
The number of larvae infected for each strain was provided on line 160 of revised manuscript.

Specific comment
19. Page 8 Biofilm formation/Figure 1. The high OD540/OD600 ratios suggest a low growth rate (thus low OD600 values). Are the differences seen in OD540/OD600 ratios due to differences in growth rate, rather than differences in biofilm formation?
Answer
In the Results section, it has been explained that “No relevant differences in growth yields were observed among the strains, thus excluding the possibility that difference in biofilm formation were due to difference in bacterial growth” (lines 197-199 of revised manuscript).

Specific comment
20. Figure 2 and supplementary table 1 are not clear. Showing the number of days that each strain survived is sufficient. Supplementary table 1 gives no additional information.
Answer
Since 23 strains are analysed for resistance to desiccation, and they show a different survival times, several experimental time-points have been included in Figure 2, with viable counts shown for each time point. We decided to show in Figure 2 the means of three experiments for each time point and for each strain, without including the standard deviation values, as they would make the Figure unreadable. However, to make possible statistical analysis of data, we reported for each strain the means ± standard deviations in the supplementary Table 1.

Specific comment
21. Figure 3. As adherence to and invasion of cells is shown, it’s more logical to show the number of adherent/invaded bacteria per cell than per well. Is it possible that the differences in invasiveness are due to differences in the susceptibility to colistin? See also comment 17.
Answer
Following reviewer’s suggestion, we have shown the number of adherent/invaded bacteria per cell in Figure 3. Because all strains included in the study were killed after a 30 min treatment with 5 mg/L of colistin, we exclude that the differences in invasiveness are due to differences in the susceptibility to colistin.
Specific comment
22. Page 10, line 226-231. ‘A similar number of bacteria adhered to…invaded epithelial cells.’ If the number of adherent bacteria is similar when the cells are incubated at 37C or at 4C, this means that the bacteria are adherent and not adherent and invaded. Therefore, the last sentence (‘since invasive bacteria…invaded epithelial cells’) can be removed.

Answer
The last sentence of the paragraph on lines 230-234 of revised manuscript was removed as correctly suggested by the Reviewer.

Specific comment

Answer
As per Reviewer’s request, the sentence on lines 236-239 of revised manuscript was rephrased.

Specific comment

Answer
“Bronchial” was replaced by “alveolar” on line 290, 329 of revised manuscript.

Specific comment

Answer
The sentence on lines 291-292 of revised manuscript was rephrased as recommended.

Specific comment
26. Page 13, line 308. Is interclonal variation meant instead of intraclonal?

Answer
Yes, the adjective “intraclonal” was replaced by “interclonal” on line 311 of revised manuscript.

Specific comment
27. Page 13, line 321, 323. Replace ‘show’ by ‘showed’.

Answer
“Show” was replaced by “showed” on line 324, 326 of revised manuscript.

Specific comment

Answer
“Since” has been removed, as recommend by the Referee.

Specific comment
29. Page 13, line 327. Replace ‘are increasingly been’ by ‘have increasingly been’ or by ‘are increasingly being’.
Answer
The sentence on line 330 of revised manuscript was corrected as suggested.

Specific comment
30. Page 23, line 514. Replace ‘5mg/ml’ by ‘5 mg/ml’.
Answer
The typo was corrected on line 515 of revised manuscript.