Reviewer's report

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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Reviewer: Anna de Breij

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This paper by Giannouli et al describes the virulence-related traits of Acinetobacter baumannii strains that belong to epidemic clonal lineages. The ability of 21 A. baumannii strains belonging to epidemic clones, including international clone I, II and III and of two susceptible A. baumannii isolates to form biofilm on plastic, resist desiccation, adhere to and invade human alveolar epithelial cells and to kill Galleria mellonella worms was compared.

Although it was already known that A. baumannii strains can form biofilm, adhere to and invade human cells, the authors nicely compare these abilities for different well-described strains of epidemic clones. Moreover, the ability of A. baumannii to kill G. mellonella contributes to the in vitro studies. Some of the results could be presented more clearly, as described below. Assessing whether biofilm formation, adherence, invasion, resistance to desiccation and in vivo virulence are associated at the strain-level would contribute to the discussion. Overall, this study gives further insight into possible virulence factors of A. baumannii.

Please find below some minor specific comments.

Minor essential revisions

1. Throughout the manuscript replace ‘adhesion and invasion of cells’ by ‘adherence to and invasion of cells’.
2. Throughout the manuscript replace ‘lethality of Acinetobacter’ by ‘the ability of Acinetobacter to kill’ or by ‘the virulence of Acinetobacter in vivo’.
3. Page 2, line 31-32. The ability to form biofilm was significantly higher for… compared to other PFGE types.
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’.
5. Page 2, line 38. What is meant with sustained biofilm growth?
7. Page 3, line 63. Replace ‘strains, initially named..’ by strains, belonging to the initially named..’
8. Page 3, line 63-64. Replace 'are biofilm-formers' by 'were biofilm-formers'.
9. Page 4, line 71-72. Replace ‘contribute to persistence’ by ‘contribute to their persistence’.
12. Page 5, line 121. Include ‘to assess the induction of biofilm formation in the presence of antibiotics, the bacteria were…’
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.
14. Page 6, line 136. Is each strain stored in a separate box? How are viable counts performed?
15. Page 6, line 140. Place reference after ‘as described previously [15], with minor modifications.’
16. Page 6, line 144. Include ‘adherent and invaded bacteria’ as this method cannot distinguish between adherent and invaded bacteria.
17. Page 7, line 148. Include ‘killing of all extracellular bacteria’. Is killing achieved within 30 minutes for all strains?
18. Page 7, line 159. Triplicate means that 30 larvae were infected with each strain?
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?
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.
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.
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.
26. Page 13, line 308. Is interclonal variation meant instead of intraclonal?
27. Page 13, line 321, 323. Replace ‘show’ by ‘showed’.
29. Page 13, line 327. Replace ‘are increasingly been’ by ‘have increasingly been’ or by ‘are increasingly being’.
30. Page 23, line 514. Replace ‘5mg/ml’ by ‘5 mg/ml’.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.