Determination of bacterial load from tissues infected with *Acinetobacter baumannii*

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**Background**

Multidrug-resistant *Acinetobacter baumannii* (*A. baumannii*) is a cause of severe infections in critically ill patients and notorious for its ability to spread epidemically. Three clonal lineages of *A. baumannii*, European (EU) clone I, II, and III, are implicated in outbreaks worldwide. Other *Acinetobacter* species, including the skin colonizer *A. junii*, are only incidentally involved in infection. Various factors are assumed to contribute to the ability of *A. baumannii* to colonize the hospital environment and patients. However, knowledge on the host’s response to *A. baumannii* is limited. Recognition by Toll-like receptor 4 and CD14 as well as early recruitment of neutrophils are important factors in the host innate defence against respiratory *A. baumannii* infection in mice.

In a previous study, we described that *A. baumannii* strains induced significantly less inflammatory cytokine production in human airway epithelial cells and cultured human macrophages *in vitro* than *A. junii* strains did. In this study we investigated the virulence of and host innate immune response to well-characterized *A. baumannii* strains, including representatives of clones I–III, and an *A. junii* strain in a mouse pneumonia model.

This protocol describes the standard procedure to determine bacterial load from mouse tissues infected with *Acinetobacter* species using the gentleMACS™ Dissociator.

**Materials and methods**

**Materials**
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS M Tubes
- Incubator (37 °C)
- Centrifuge
- Phosphate-buffered saline (PBS), pH 7.4
- Blood agar or diagnostic sensitivity test (DST) agar

**Methods**
1. Remove the spleen and lung from the mouse.
2. Weigh organs.
3. Transfer the whole desired organ into the gentleMACS M Tube containing 3 mL of PBS.
4. Tightly close the M Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program **m_spleen_01**.
6. Centrifuge M Tubes for 5 minutes at 2000×g to remove cell debris.
7. Serially dilute the homogenates using PBS.
8. Spot 50 µL of each dilution on blood agar or DST agar plates.
9. Incubate plates overnight at 37 °C.
10. Determine CFU counts.
Results

We found a striking difference in morbidity and mortality associated with *A. baumannii* strains, with EU clone I and II strains being the most virulent. Differences in mortality between the strains could not be attributed to bacterial loads in lungs or blood, suggesting that proliferation in lungs and extrapulmonary dissemination are not the only factors contributing to the virulence of these strains. Furthermore, the outcome of experimental *A. baumannii* pneumonia was associated with IL-10 and IL-12p40/IL-23 levels. Future studies will have to clarify whether this response influences the impact of *A. baumannii* strains in the human host. If so, levels of these mediators may have predictive values or be targets for treatment.

Conclusion

Bacterial load determination of *Acinetobacter* species in mice organs can be accomplished with ease using the gentleMACS Dissociator.

Reference


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Figure 1: CFU/g of lung tissue 1–4 days after infection with the different *Acinetobacter* strains.