**The effect of pleural fluid on citrate synthase expression and the link with *A. baumannii* survival**

JasmineMartinez1, Robert Courville1,Parvin Shahrestani1­­, Robert A. Bonomo2,3,4, Rodrigo Sieira5, Maria Soledad Ramirez1\*.

1Center for Applied Biotechnology Studies, Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California, USA, 2Medical Service and GRECC, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA, 3Departments of Medicine, Pharmacology, Molecular Biology and Microbiology, Biochemistry, Proteomics and Bioinformatics, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA, 4CWRU-Cleveland VAMC Center for Antimicrobial Resistance and Epidemiology (Case VA CARES), Cleveland, Ohio, USA, 5Fundación Instituto Leloir – IIBBA CONICET, Buenos Aires, Argentina.

**Running Title:** PF dictates *gltA* expression for survival in *A. baumannii*

**Keywords:** *Acinetobacter baumannii*, human pleural fluid, survival, metabolism, transcriptomic analysis, citrate synthase

**\*Corresponding author.**

María Soledad Ramírez, PhD.

Assistant Professor

Dept. Biological Science

California State University Fullerton

800 N State College Blvd

Fullerton, CA 92831

e-mail: [msramirez@fullerton.edu/](mailto:msramirez@fullerton.edu/) Tel: +1 657-278-4562

*Acinetobacter baumannii* is a nosocomial pathogen that is frequently resistant to multiple antibiotics and can cause pneumonia, bacteremia and wound infections with associated high mortality rates. The Centers for Disease Control and Prevention, CDC, 2019 Antibiotic Resistance Threats Report moved carbapenem resistant *A. baumannii* (CRAB) into the urgent threat category 1. Intrinsic features of *A. baumannii*, such as its ability to persist in environmental and clinical settings for long periods of time and the capacity to acquire foreign DNA, have contributed to the success of *A. baumannii* as a major nosocomial pathogen (ref).

Studies exposing *A. baumannii* to different human products showed that *A. baumannii* can respond to these stimuli shaping its pathogenic behavior 2. *A. baumannii* is also able to undergo changes in its metabolism and nutritional needs under unfavorable conditions. This metabolic flexibility is shown to overcome host-imposed nutrient limitation through induction of expression of metal uptake systems, and transcriptional regulators 3.

Recent findings demonstrated that when *A. baumannii* is exposed to pleural fluid (PF), a body secretion which primarily functions to lubricate the lung pleurae during respiratory movements, a large number of genes related with metabolic processes were affected 4. More than 55% of the genes differentially expressed were associated with altered metabolic processes, suggesting that the metabolic versatility affected the survival and persistence of *A. baumannii* within the host. In the presence of PF, we observed changes in intracellular pyruvate and phenylalanine metabolism that enhanced *A. baumannii’s* cytotoxicity and immune evasion 4. When exposed to PF, *A. baumannii* respond by modifying the expression of genes associated with pathogenicity and adaptative response to stress 5. A considerable number of genes (1120 considering log2 > 1 with adjusted *P*-value < 0.05) are affected in the presence of PF, suggesting that a multifactorial strategy is involved in scenarios containing stressors and detrimental conditions that lead to *A. baumannii* adaptation and success in harsh conditions.

It has been well studied that pathogens need to overcome nutrient limitation, such as iron, to colonize and survive within the host. Most of the pathogens possess a variety of proteins and molecules to harvest sequestered iron 6.During inflammatory diseases, such as those that result in pleural effusion, the neutrophil gelatinase-associated lipocalin (NGAL) is significantly released by neutrophils 7. NGAL, also known as Lipocalin 2 (Lcn2), is known to sequester iron and also possesses immunomodulatory effects 8. It was shown that components present in PF, such as macrophages, lymphocytes, monocytes, granulocytes, proteins, LDH, and low concentrations of essential metals, are triggering an adaptive response in *A. baumannii* 4,5.

While investigating an unknown Lcn2-dependent factors that could be playing a role in evasion of nutritional immunity during *Klebsiella pneumoniae* infection, Vornhagen et al. 2019 showed that the citrate synthase, GltA, plays a critical role in *Klebsiella pneumoniae* infection by demonstrating how, the metabolic flexibility influences bacterial fitness 10. They observed that GltA allows *K. pneumoniae* to replicate in the lung and intestine by using diverse nutrients. The citrate synthase gene, *gltA*, showed to be critical during *K. pneumoniae* infections, enabling this pathogen to grow in the lung and digestive tract by enhancing its ability to utilize various nutrients 9.

Considering our previous collected data when *A. baumannii* exposed to PF, we hypothesize that the citrate metabolism, where *gltA* is involved, is playing a role in *A. baumanii’s* metabolic flexibility to overcome nutrient deficiency and stress to succeed in diverse niches. Here, we focus our studies on *gltA* and its potential role on *A. baumannii*’s metabolic flexibility to survive under stressful conditions.

Observing our previous transcriptomic data, we found that *gltA* was upregulated under PF treatment (log2 fold change of 2.14). In addition, other genes of the TCA were up or down-regulated (Fig. 1A). To first identify *A. baumannii’s* capacity to use different carbon and nitrogen sources upon exposure to PF, and if its ability to use diverse nutrients supports its growth in this condition, phenotype microarrays of different carbon sources and nitrogen sources were analyzed. Our data showed that under PF treatment, *A. baumannii* had increased growth in L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, L-Methionine, and L-Valine suggesting that *A. baumannii* can utilize different nutrients to promote its growth within a stressful environment.

Considering that citrate is known to be an iron carrier, and knowing that PF is an ion limited media, we analyzed the iron-associated genes under PF treatment, and we observed that 23 DEGs are downregulated (Fig. 1B). This can be suggesting that citrate can be acting as an iron carrier to overcome the limiting condition of this metal.

To assess if the presence of PF in *A. baumannii’s* growth environment would increase virulence, we carried out infection assays in a *Drosophila melanogaster* model of infection. A recent paper proposed *Drosophila* as a novel model system to study *A. baumannii* interaction with host cells 10. Our results showed that when *A. baumannii* was exposed to PF, the survival of the flies was reduced to 95.1% of inoculated individuals (Fig. 1C, *P*-value= 0.15). Under PF, both, *A. baumannii’s* metabolic flexibility and increase transcriptional response, allow this pathogen to conquer different niches. It is noteworthy that the *D. melanogaster* population used in this study is outbred and has been shown to be more robust than inbred fly lines used in many laboratories 11. Thus, even in highly robust *D. melanogaster* populations, an increase in virulence of *A. baumannii* when exposed to PF was observed.

Collectively, our data shows that metabolic flexibility and metabolites can serve as tools for opportunistic pathogens to overcome stress or limited nutrient conditions to persist and survive in different sites of infection. Future in vivo infection studies in appropriate models will reinforce and validate the observe response.

**Funding:** The authors’ work was supported by NIH SC3GM125556 to MSR, R01AI100560, R01AI063517, R21AI114508, and R01AI072219 to RAB. This study was supported in part by funds and/or facilities provided by the Cleveland Department of Veterans Affairs, Award Number 1I01BX001974 to RAB from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development and the Geriatric Research Education and Clinical Center VISN 10 to RAB. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Veterans Affairs. JM has a McNair Scholar Fellowship

**Transparency declarations.** None to declare.

**Reference**

1 CDC. Antibiotic Resistance Threats in the United States. *Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019* (2019).

2 Murray, G. L. *et al.* Global Gene Expression Profile of *Acinetobacter baumannii* During Bacteremia. *J Infect Dis* **215**, S52-s57, doi:10.1093/infdis/jiw529 (2017).

3 Juttukonda, L. J., Chazin, W. J. & Skaar, E. P. *Acinetobacter baumannii* Coordinates Urea Metabolism with Metal Import To Resist Host-Mediated Metal Limitation. *MBio* **7**, doi:10.1128/mBio.01475-16 (2016).

4 Rodman Nyah, M. J., Fung Sammie, Nakanouchi Jun, Myers Amber L., Harris Caitlin M., Dang Emily, Fernandez Jennifer S., Liu Christine, Mendoza Anthony M., Jimenez Veronica, Nikolaidis Nikolas, Brennan Catherine A., Bonomo Robert A., Sieira Rodrigo, Ramirez Maria Soledad. Human Pleural Fluid Elicits Pyruvate and Phenylalanine Metabolism in *Acinetobacter baumannii* to Enhance Cytotoxicity and Immune Evasion *Frontiers in microbiology* **10**, 1581 doi:10.3389/fmicb.2019.01581 (2019).

5 Martinez, J. *et al.* Human pleural fluid triggers global changes in the transcriptional landscape of *Acinetobacter baumannii* as an adaptive response to stress. *Sci Rep* **9**, 17251, doi:10.1038/s41598-019-53847-2 (2019).

6 Hood, M. I. & Skaar, E. P. Nutritional immunity: transition metals at the pathogen–host interface. *Nature Reviews Microbiology* **10**, 525-537, doi:10.1038/nrmicro2836 (2012).

7 Gümüs, A. *et al.* A novel biomarker in the diagnosis of parapneumonic effusion: neutrophil gelatinase-associated lipocalin. *Multidisciplinary Respiratory Medicine* **9**, 49, doi:10.1186/2049-6958-9-49 (2014).

8 Holden, V. I. *et al.* Bacterial siderophores that evade or overwhelm lipocalin 2 induce hypoxia inducible factor 1alpha and proinflammatory cytokine secretion in cultured respiratory epithelial cells. *Infect Immun* **82**, 3826-3836, doi:10.1128/iai.01849-14 (2014).

9 Vornhagen, J. *et al.* The *Klebsiella pneumoniae* citrate synthase gene, gltA, influences site specific fitness during infection. *PLoS Pathog* **15**, e1008010, doi:10.1371/journal.ppat.1008010 (2019).

10 Qin, Q.-M. *et al.* A tractable *Drosophila* cell system enables rapid identification of *Acinetobacter baumannii* host factors. *bioRxiv*, 2020.2004.2009.034157, doi:10.1101/2020.04.09.034157 (2020).

11 Rose M.R., P. H. B., and M. Matos. *A Case Study in the Evolution of Aging*. (World Scientific Publishing, 2014).

**Figure Legend**

**Figure 1.*****A. baumannii* can utilize different carbon and nitrogen sources to help it survive in stressful environments.** A) The TCA cycle associated genes in *A. baumannii* strain A118 were analyzed when it was exposed to PF. Phenotype microarrays were conducted with strain A118s to identify which sources *A. baumannii* could use in order to support its growth in PF. B) A heatmap of iron-uptake associated genes when *A. baumannii* strain A118 was exposed to 4% PF. C) Percent survival of *D. melanogaster* infected with *A. baumannii* strain A118 in the presence or absence of 4% PF.