Human Serum Albumin (HSA) Regulates the Expression of Histone-like Nucleoid Structure Protein (H-NS) in *Acinetobacter baumannii*

Jenny Escalante

Center for Applied Biotechnology Studies, , Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California

Brent Nishimura

Center for Applied Biotechnology Studies, , Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California

Marisel R. Tuttobene

Área Biología Molecular, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

Tomás Subils

Instituto de Procesos Biotecnológicos y Químicos de Rosario (IPROBYQ, CONICET-UNR), Rosario

Camila Pimentel

Center for Applied Biotechnology Studies, , Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California

Nardin Georgios

Center for Applied Biotechnology Studies, , Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California

Rodrigo Sieira

Fundación Instituto Leloir – IIBBA CONICET, Buenos Aires

Robert A. Bonomo

Research Service and GRECC, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio

Marcelo E. Tolmasky

Center for Applied Biotechnology Studies, , Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California

María Soledad Ramírez (✉ msramirez@fullerton.edu)

Center for Applied Biotechnology Studies, , Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California

Keywords: Acinetobacter baumannii, H-NS, stress, human serum albumin, biofilm, quorum sensing

Posted Date: February 16th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1293237/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Acinetobacter baumannii, a common cause of nosocomial infection, easily adapts to multiple environments. These characteristics together with this bacterium's ability to acquire antibiotic resistance determinants make it a notorious pathogen. The presence of human serum albumin (HSA) is associated to modification in levels of expression of numerous genes. The presence of HSA in the culture medium is also correlated with a reduction in levels of the global suppressor histone-like nucleoid structure protein, H-NS. Transcriptome analysis using a wild type and isogenic Δhns strains cultured in lysogeny broth, LB, in the presence or absence of HSA revealed that a subset of eleven, out of 183 genes whose expression is modified by HSA, were regulated through H-NS. Six and five of these genes were up- or down-regulated, respectively. Three of these genes have functions in quorum sensing (*acdA*, *kar* and *fadD*), one in quorum quenching (*aidA*), two in stress response (*katE*, *ywrO*), three in metabolism (*phaC*, *yedL1*, and *yedL2*), one in biofilm formation (*csuAB*), and one in β -oxidation of fatty acids (*fadA*). The regulation of these genes was assessed by: *i*) transcriptional analysis and qPCR at the transcriptional level; and *ii*) by determining the phenotypic characteristics of each function. The results of these studies support the hypothesis that HSA-mediated reduction of H-NS levels may be one very important regulatory circuit utilized by *A. baumannii* to adapt to selected environments, such as those where HSA-containing human fluids are abundant.

Introduction

Acinetobacter baumannii has emerged as a significant nosocomial pathogen as it is associated with high levels of morbidity and mortality (1–5). The majority of strains recovered from patients in the hospital are multidrug resistant (MDR), a characteristic that permitted the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) to classify this pathogen as an “urgent threat” (6, 7).

A. baumannii strains are genetically diverse as a result of their extraordinary capacity to naturally acquire DNA via transformation (8–13). Recent studies show that human serum albumin (HSA), a major blood protein, enhances transformation frequency and increases expression levels of type IV pilus-associated genes in *A. baumannii* (9, 14, 15). Moreover, the effect of HSA is not limited to these functions; HSA also affects expression of genes involved in motility, biofilm formation, efflux pumps, metabolism, capsule synthesis, transcriptional regulation, antibiotic resistance, and pathogenesis as determined by transcriptomic analysis (14). These studies also reveal that the *A. baumannii* *hns* (histone-like nucleoid structuring) gene expression levels decrease in cells exposed to purified HSA. An attractive hypothesis to explain a physiological role from these observations is that there is a regulatory circuit that links HSA to reduced H-NS protein intracellular concentrations, which affect expression of selected genes helping *A. baumannii*'s adaptation to HSA-containing human fluids (14).

In *A. baumannii* the H-NS protein, a global transcriptional repressor in many Gram-negative bacteria (16–19), plays a major role in persistence and expression of virulence-associated genes (17, 20), stress induced by carbapenemase expression (21), as well as expression of genes associated to antibiotic

resistance, biofilm formation, and quorum sensing (22). H-NS may also be necessary for natural competence in *A. baumannii* as an *hns* null mutant showed significantly lower expression levels of genes related to acquisition of DNA from the environment (23). To better understand the correlation of the effects produced by extracellular HSA and intracellular H-NS, we studied the effect of the presence of HSA in the milieu on the expression H-NS and other *A. baumannii*'s genes. Comparative transcriptomic analyses (RNA-seq) using *A. baumannii* AB5075 and AB5075 Δhns cultured in the presence or absence of HSA revealed significant modifications in levels of expression of specific genes. These experiments, in combination with phenotypic assays, indicate that HSA modifies expression levels of some *A. baumannii* genes through regulating expression of H-NS.

Results And Discussion

Transcriptional effect of HSA on gene expression

Transcriptome analysis of *A. baumannii* AB5075 and AB5075 Δhns cultured in LB broth revealed a differential expression profile of 183 genes (22). When *A. baumannii* AB5075 was cultured in LB broth with or without HSA there were 30 genes differentially expressed (Table S1). In both cases, an FDR-adjusted *P-value* of < 0.05 and $\log_2$ -fold change > 1 was considered as the differentially expressed-genes (DEGs). Inspection of the DEGs in both analyses showed that there were 11 genes in common (hhDEG) (Fig. 1A and Table S2). These genes are suitable candidates to be regulated by the presence of HSA through modification of the intracellular H-NS concentration. Six and five of these DEGs were up- or down-regulated, respectively (Fig. 1B). Three of these genes are involved in quorum sensing (*acdA*, *kar* and *fadD*), and one in quorum quenching (*aidA*) (24). Other hhDEGs are associated with biofilm formation (*csuAB*) (25), β -oxidation of fatty acid (*fadA*) (26), stress response (*katE*, *ywrO*) (27), and metabolism (*phaC*, *yedL1*, and *yedL2*) (Fig. 1B).

Cultures of the multidrug resistant, hypervirulent *A. baumannii* AB5075 model strain in the presence or absence of a physiological concentration of HSA were next subjected to quantitative RT-PCR (qRT-PCR) using total RNA to confirm that the presence of HSA in the growth medium results in a reduction of the H-NS synthesis (Fig. 1C).

We next determined the levels of H-NS in *A. baumannii* AB5075 pMBLe-*hns*, a strain that carries a plasmid that overexpresses H-NS. Fig. S1 shows that expression was elevated in cells grown in LB medium, but the H-NS levels were drastically reduced when the medium was supplemented with 3.5% HSA. This strain and the wild type *A. baumannii* AB5075 were used to assess the expression of nine selected hhDEGs by qRT-PCR using RNA obtained from cultures carried out in LB broth supplemented with and without 3.5% HSA. Expression of *acdA*, *kar*, *fadD*, and *aidA* were upregulated in *A. baumannii* AB5075 grown in the presence of HSA. However, it was of interest that increasing the concentration of H-NS did not modify the expression of these genes (compare bar AB5075LB with AB5075 pMBLe-*hns* in Fig. 2). Expression of *csuAB* was downregulated in *A. baumannii* AB5075 growing in the presence of HAS.

In contrast, the *fadA* gene behaved unexpectedly; significant changes were not observed in the presence or absence of HSA in *A. baumannii* AB5075 and a reduction in expression was observed in the overproducing strain that was driven by the presence of HSA, i.e., when overexpression of H-NS was reversed (Fig. 2). The molecular basis of these modifications and changes are unknown at this time. The stress-response related genes *katE* and *ywrO* were downregulated in the presence of HSA and differences were not noted when H-NS was overexpressed (Fig. 2). The metabolic gene *phaC* a key enzyme in the polymerization of polyhydroxyalkanoates (PHAs), showed a significant up-regulation in both strains, but significant modifications to levels of expression when H-NS was overexpressed was not seen (Fig. 2).

Since biofilm and quorum sensing are intimately related to *A. baumannii*'s virulence (and we identified genes involved in these two processes that are regulated by HSA and H-NS), we expanded the qRT-PCR analysis to two other genes associated with these processes. The expression of *csuE*, associated to biofilm formation, was downregulated when *A. baumannii* AB5075 was cultured in the presence of HSA. In *A. baumannii* AB5075 pMBLe-*hns* was overexpressed; but a significant downregulation was observed when HSA was present in the culture medium (Fig. S2). In the case of *abaI*, which codes for the acyl homoserine lactone (AHL) synthase, the presence of HSA in the milieu produced a significant reduction in expression. *A. baumannii* AB5075 pMBLe of H-NS growing in the presence or absence of HSA expressed the gene at low levels (Fig. S2).

HSA plays a role in modulating the biofilm formation, the quorum sensing network, and the oxidative stress through H-NS

A. baumannii AB5075 grown in the presence of HSA produced a reduced mass of biofilm, which was similar to that produced by the *hns* deficient mutant (Fig. 3A and B). The *A. baumannii* AB5075 pMBLe-*hns* strain as well as the mutant complemented by the plasmid pMBLe-*hns* cultured in the absence of HSA showed elevated levels of biofilm production. In *A. baumannii* AB5075 pMBLe-*hns* a regulatory effect was noted in HSA-containing medium. Taken together, these results support a regulatory role of HSA through modifying H-NS expression.

Phenotypic modifications in quorum sensing were assessed determining levels of acyl-homoserine lactone (AHL) using the *Agrobacterium tumefaciens*-based solid plate assays (28, 29). Rodgers et al. demonstrated that the supernatants from *A. baumannii* AB5075 or AB5075 Δhns produced similar intensity of color, likely caused by an increase in both AHL synthesis (quorum sensing) and lactonase activity (quorum quenching) in AB5075 Δhns strain (22). To study the effect of HSA on AHL secretion into the surrounding medium, *A. baumannii* AB5075, AB5075 Δhns , and the strains complemented with pMBLe-*hns* were cultured in different conditions before assessing the AHL concentration in the growth medium. *A. baumannii* AB5075 produced much lower amount of AHL when it was cultured in the presence of HSA (Fig. S3). *A. baumannii* AB5075 pMBLe-*hns* growing in the presence or absence of HSA and *A. baumannii* AB5075 Δhns pMBLe-*hns* cultured in LB broth, all produced low levels of AHL. The low AHL concentration in the growth medium could be explained by cancelling modifications in expression of

genes associated with AHL synthesis (*acdA*, *kar*, *fadD*, and *abaI*) and lactonase, which is responsible for quorum quenching activity (*aidA*) (Fig. 1, 2, and S2).

Hydrogen peroxide is a disinfectant with potent bactericidal activity that is used in vaporized form to control outbreaks of multi-resistant *A. baumannii* infections (30) (31, 32). Since *A. baumannii* is catalase-positive and the *katE* gene was a hhDEG, down-regulated in the presence of HSA, we assessed the production of catalase activity, as the decrease in absorbance at 240 nm resulting from the consumption of H₂O₂ (Fig. 4). Addition of HSA to the growth medium resulted in a reduction of catalase activity in the wild type strain. Equally low activity was produced by *A. baumannii* AB5075 Δhns growing in the absence of added HSA, which suggest that the action of HSA occurs through reduction of H-NS synthesis (Fig. 4). These results agreed with both RNA-seq analyses and RT-qPCR results. As expected, the complemented *A. baumannii* AB5075 Δhns pMBLe-*hns* produced higher catalase activity when compared to *A. baumannii* AB5075 (Fig. 4). Surprisingly, overexpressing *hns* showed reduced catalase activity when compared to the wild type strain; we still do not know the molecular bases for this unexpected results (Fig. 4).

The results described in this section support the hypothesis that some *A. baumannii* genes are regulated by H-NS, whose expression is modified by the presence of HSA in the milieu. H-NS is a nucleoid-associated protein that binds DNA in a relatively nonspecific manner (it shows preference for AT-rich and curved regions) and alters its topology, which modifies levels of transcription. The effects of H-NS in *A. baumannii* have not been thoroughly studied (17, 20, 22). An early analysis found that an *A. baumannii* mutant containing a disrupted *hns* gene was modified in the expression of several virulence genes such as those associated with the autotransporter Ata, the type VI secretion system, a type I pilus cluster, the acetoin metabolism, and phenylacetic acid degradation (17). This strain also showed altered adherence to biotic surfaces and increase virulence as determined using the *Caenorhabditis elegans* infection model system (17). A previous study showed that exposure of the model *A. baumannii* A118 to HSA was accompanied by a reduction of expression of H-NS and a wide variety of genes related to antibiotic resistance and stress response as well as genes that encode functions associated with natural competence (14, 22, 23, 33).

The global nature of H-NS as transcriptional regulator makes it challenging to definitely associate genes that are regulated by H-NS through variations in levels of expression of the regulator when the cells are exposed to HSA. However, despite these limitations, the results described in this work strongly suggest that there is a group of genes whose expression is indirectly regulated at the transcriptional level by HSA through reducing H-NS expression. We conclude that HSA-mediated reduction of H-NS levels may be one regulatory circuit utilized by *A. baumannii* to adapt to selected environments such as those where HSA-containing human fluids are abundant.

Conclusions

H-NS is a highly abundant intracellular protein that functions as a nucleoid organizer and a transcriptional silencer. In this work we gathered evidence that supports the role of HSA and H-NS in

regulating quorum sensing and quorum quenching, biofilm, β -oxidation, stress, and metabolism related genes. Future analysis planned using chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), in wild-type or an *hns* mutant, in the presence or absence of HSA will contribute to new insights into the molecular mechanisms governing the roles of HSA and H-NS in regulating the genes identified in this work and most probably other genes involved in the mechanisms of persistence and pathogenicity of *A. baumannii*.

Materials And Methods

Bacterial strains. The multidrug and hypervirulent AB5075 strain (34), its isogenic *hns* mutant (AB5075 Δhns) (35) and AB5075 Δhns pMBLe-*hns* (22) were used in the present study.

Electroporation. Electro-competent *A. baumannii* AB5075 cells were prepared and mixed with 25 ng of pMBLe-*hns* plasmid DNA (containing apramycin resistance) followed by electroporation with a Bio-Rad Gene Pulser instrument at 2.5 kV, 200 Ω , 25 μ F. The electroporated cells were placed in recovery with 1 ml of LB broth for 2 h at 37°C in a shaking incubator followed by culturing overnight at 37°C on LB agar containing 15 μ g/ml apramycin. At least 10 colonies were picked to confirm the presence of the different plasmids. To confirm their presence, plasmid extraction followed by gel electrophoresis analysis.

RNA extraction and RNA-seq analysis. *A. baumannii* AB5075 and derivative strains were cultured in LB broth and incubated with agitation for 18 h at 37°C. Overnight cultures were then diluted 1:10 in fresh LB broth or LB broth supplemented with HSA and incubated with agitation for 7 h at 37°C. The Direct-zol RNA Kit (Zymo Research, Irvine, CA, USA) was used to perform the RNA extraction in triplicates. RNA samples were DNase treated (Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instruction. Samples were confirmed to have no DNA contamination through PCR amplification of the 16S rDNA gene. RNA sequencing was outsourced to Novogene (Novogene Corporation, Sacramento, CA, USA). Ribosomal RNA-depletion was done using the Ribo-Zero kit (Illumina) and the construction of the cDNA library was performed with the TruSeq Stranded Total RNA Library Prep kit (Illumina) from three independent replicates per sample. Analysis of the quality of the illumina reads, trimming of low-quality bases and removal of Illumina adapters was performed as described previously (36). Reads were aligned to the genome of *A. baumannii* AB5075 using Burrows-Wheeler Alignment (BWA) software (v0.7.17) BWA and visualized using the Integrative Genomics Viewer (IGV). Read counts per gene were calculated using FeatureCounts (37), and differential expression analysis was performed using DESeq2. Differentially expressed genes (DEGs) were defined as those displaying an FDR adjusted *P-value* of < 0.05 and log₂ fold change > 1. In the present work, previously published RNA-seq reads (GEO accession No GSE167117) corresponding to *A. baumannii* AB5075 vs AB5075 Δhns incubated in LB broth (22) or *A. baumannii* AB5075 grown in LB broth vs grown in 0.2% HSA were analyzed.

qRT-PCR was performed to confirm and analyze the expression genes. cDNA was prepared using the iScript Reverse Transcription Supermix for qRT-PCR (BioRad, Hercules, CA, USA) and quantitative PCR was performed using iQ™ SYBR Green Supermix (BioRad, Hercules, CA, USA) per the manufacturer's

recommendations, respectively. Results were analyzed using the $2^{-\Delta\Delta C_t}$ method(38) in which *rpoB* was used as control as the control gene. Experiments were performed in technical and biological triplicates. The results from experiments performed were statistical analyzed (ANOVA followed by Tukey's multiple comparison test) using GraphPad Prism (GraphPad software, San Diego, CA, USA). A *P-value* < 0.05 was considered significant.

The RNA-seq data analysed during the current study are available in the NCBI repository with the GEO accession No GSE167117.

Catalase activity measurements. Crude extract of *A. baumannii* AB5075 and derivate strains cultured in LB or LB supplemented with 3.5% HSA were utilized to determinate the catalase activity spectrophotometrically by monitoring the decrease in absorbance at 240 nm resulting from the consumption of H₂O₂ using a UV visible spectrophotometer (SpectraMax M3)(39).

Biofilm assay. *A. baumannii* AB5075 and derivative strains were cultured in fresh LB medium, or LB supplemented with 3.5% has with agitation for 18 h at 37 °C. Tubes were emptied, washed three times with 1X phosphate-buffered saline (PBS) and stained with 1% crystal violet (CV) for 15 m. Excess CV was removed by washing three more with 1X PBS. Experiments were performed in triplicate, with at least three technical replicates per biological replicate.

N-acyl homoserine lactone (AHL) detection. *Agrobacterium tumefaciens*-based solid plate assays were carried out to detect N-Acyl Homoserine Lactone (AHL) production (40) as described in previous work(28).Initially, 500 µL of the homogenate were loaded in a central well of 0.7% LB agar plates supplemented with 40 µg of 5-bromo-3-indolyl-β-D-galactopyranoside (X-Gal) per mL and 250 µL (OD = 2.5) of the overnight culture of *Agrobacterium tumefaciens* biosensor. The presence of AHL was determined by the development of blue coloring (29). As a positive control, 100 µL of N-Decanoyl-dl-homoserine lactone (C10-AHL) 12.5 mg/mL was utilized. Quantification of 5,5'-dibromo-4,4'-dichloro-indigo production in different conditions was determined using ImageJ software (NIH) by measuring the intensity of each complete plate, and subtracting the intensity measured in the negative control. The values were normalized to the positive control, which received the arbitrary value of 100.

Statistical analysis. Experiments performed at least in triplicates were statistically analyzed by ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism (GraphPad software, San Diego, CA, USA). A *P-value* < 0.05 was considered significant.

All procedures performed in this study were in accordance with the CSUF Institutional Biosafety Committee Approval plan (DBH117-01) and follow the NIH, CDC, OSHA and other environmental and occupational regulations.

Declarations

Author contributions

J.E., B.N., M.R.T., T.S., C.P., N.G., R.S., R.A.B, M.E.T. and M.S.R. conceived the study and designed the experiments. J.E., B.N., M.R.T., T.S., C.P., N.G., R.S., and M.S.R. performed the experiments and genomics and bioinformatics analyses. J.E., B.N., M.R.T., T.S., C.P., N.G., R.S., R.A.B., M.E.T. and M.S.R. analyzed the data and interpreted the results. R.A.B., M.E.T. and M.S.R. contributed reagents/materials/analysis tools. M.R.T., T.S., R.S., R.A.B., M.E.T. and M.S.R. wrote and revised the manuscript. All authors read and approved the final manuscript.

Funding

The authors' work was supported by NIH SC3GM125556 to MSR, R01AI100560 to RAB, R01AI063517, R01AI072219 to RAB and 2R15 AI047115 to MET. JE and CP were supported by Grant MHRT 2T37MD001368 from the National Institute on Minority Health and Health Disparities, National Institute of Health. 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. MRT and TS are recipient of a postdoctoral fellowship from CONICET. R.S. is a staff member from CONICET.

Competing interests

The authors declare no competing interests.

Data availability statement: The datasets generated and analyzed during the current study are available in the Gene Expression Omnibus (GEO) repository, (GEO accession No GSE167117).

References

1. Ramirez MS, Bonomo RA, Tolmasky ME. 2020. Carbapenemases: Transforming *Acinetobacter baumannii* into a Yet More Dangerous Menace. *Biomolecules* 10.
2. Paterson DL, Bonomo RA. 2019. Multidrug-resistant gram-negative pathogens: the urgent need for 'old' polymyxins. *Adv Exp Med Biol* 1145:9–13.
3. Spellberg B, Bonomo RA. 2014. The deadly impact of extreme drug resistance in *Acinetobacter baumannii*. *Crit Care Med* 42:1289–91.
4. Spellberg B, Bonomo RA. 2013. "Airborne assault": a new dimension in *Acinetobacter baumannii* transmission*. *Crit Care Med* 41:2042–4.
5. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 51:3471–84.
6. CDC. 2019. Antibiotic Resistance Threats in the United States. Atlanta, GA: US Department of Health and Human Services, CDC; 2019.

7. (WHO). WHO. 2017. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics.
8. Vesel N, Blokesch M. 2021. Pilus production in *Acinetobacter baumannii* is growth phase dependent and essential for natural transformation. *J Bacteriol*.
9. Traglia GM, Quinn B, Schramm ST, Soler-Bistue A, Ramirez MS. 2016. Serum Albumin and Ca²⁺ Are Natural Competence Inducers in the Human Pathogen *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 60:4920–9.
10. Touchon M, Cury J, Yoon EJ, Krizova L, Cerqueira GC, Murphy C, Feldgarden M, Wortman J, Clermont D, Lambert T, Grillot-Courvalin C, Nemec A, Courvalin P, Rocha EP. 2014. The genomic diversification of the whole *Acinetobacter* genus: origins, mechanisms, and consequences. *Genome Biol Evol* 6:2866–82.
11. Ramirez MS, Merkier AK, Quiroga MP, Centron D. 2012. *Acinetobacter baumannii* is able to gain and maintain a plasmid harbouring *Int35* found in *Enterobacteriaceae* isolates from Argentina. *Curr Microbiol* 64:211–3.
12. Martinez J, Liu C, Rodman N, Fernandez JS, Barberis C, Sieira R, Perez F, Bonomo RA, Ramirez MS. 2018. Human fluids alter DNA-acquisition in *Acinetobacter baumannii*. *Diagn Microbiol Infect Dis*.
13. Domingues S, Rosario N, Candido A, Neto D, Nielsen KM, Da Silva GJ. 2019. Competence for Natural Transformation Is Common among Clinical Strains of Resistant *Acinetobacter* spp. *Microorganisms* 7.
14. Quinn B, Rodman N, Jara E, Fernandez JS, Martinez J, Traglia GM, Montana S, Cantera V, Place K, Bonomo RA, Iriarte A, Ramirez MS. 2018. Human serum albumin alters specific genes that can play a role in survival and persistence in *Acinetobacter baumannii*. *Sci Rep* 8:14741.
15. Le C, Pimentel C, Tuttobene MR, Subils T, Nishimura B, Traglia GM, Perez F, Papp-Wallace KM, Bonomo RA, Tolmasky ME, Ramirez MS. 2021. Interplay between meropenem and human serum albumin on expression of carbapenem resistance genes and natural competence in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*:AAC0101921.
16. Wang H, Ayala JC, Benitez JA, Silva AJ. 2015. RNA-seq analysis identifies new genes regulated by the histone-like nucleoid structuring protein (H-NS) affecting *Vibrio cholerae* virulence, stress response and chemotaxis. *PLoS One* 10:e0118295.
17. Eijkelkamp BA, Stroehrer UH, Hassan KA, Elbourne LD, Paulsen IT, Brown MH. 2013. H-NS plays a role in expression of *Acinetobacter baumannii* virulence features. *Infect Immun* 81:2574–83.
18. Prosseda G, Fradiani PA, Di Lorenzo M, Falconi M, Micheli G, Casalino M, Nicoletti M, Colonna B. 1998. A role for H-NS in the regulation of the *virF* gene of *Shigella* and enteroinvasive *Escherichia coli*. *Res Microbiol* 149:15–25.
19. Hurtado-Escobar GA, Grepinet O, Raymond P, Abed N, Velge P, Virlogeux-Payant I. 2019. H-NS is the major repressor of *Salmonella Typhimurium* Pef fimbriae expression. *Virulence* 10:849–867.
20. Deveson Lucas D, Crane B, Wright A, Han ML, Moffatt J, Bulach D, Gladman SL, Powell D, Aranda J, Seemann T, Machado D, Pacheco T, Marques T, Viveiros M, Nation R, Li J, Harper M, Boyce JD. 2018.

- Emergence of High-Level Colistin Resistance in an *Acinetobacter baumannii* Clinical Isolate Mediated by Inactivation of the Global Regulator H-NS. *Antimicrob Agents Chemother* 62.
21. Huang F, Fitchett N, Razo-Gutierrez C, Le C, Martinez J, Ra G, Lopez C, Gonzalez LJ, Sieira R, Vila AJ, Bonomo RA, Ramirez MS. 2020. The H-NS Regulator Plays a Role in the Stress Induced by Carbapenemase Expression in *Acinetobacter baumannii*. *mSphere* 5.
  22. Rodgers D, Le C, Pimentel C, Tuttobene MR, Subils T, Escalante J, Nishimura B, Vescovi EG, Sieira R, Bonomo RA, Tolmasky ME, Ramirez MS. 2021. Histone-like nucleoid-structuring protein (H-NS) regulatory role in antibiotic resistance in *Acinetobacter baumannii*. *Sci Rep* 11:18414.
  23. Le C, Pimentel C, Tuttobene MR, Subils T, Escalante J, Nishimura B, Arriaga S, Rodgers D, Bonomo RA, Sieira R, Tolmasky ME, Ramírez MS. 2021. Involvement of the Histone-Like Nucleoid Structuring Protein (H-NS) in. *Pathogens* 10.
  24. Lopez M, Mayer C, Fernandez-Garcia L, Blasco L, Muras A, Ruiz FM, Bou G, Otero A, Tomas M, Geih G. 2017. Quorum sensing network in clinical strains of *A. baumannii*: AidA is a new quorum quenching enzyme. *PLoS One* 12:e0174454.
  25. Tomaras AP, Flagler MJ, Dorsey CW, Gaddy JA, Actis LA. 2008. Characterization of a two-component regulatory system from *Acinetobacter baumannii* that controls biofilm formation and cellular morphology. *Microbiology* 154:3398–3409.
  26. Jiang JH, Hassan KA, Begg SL, Rupasinghe TWT, Naidu V, Pederick VG, Khorvash M, Whittall JJ, Paton JC, Paulsen IT, McDevitt CA, Peleg AY, Eijkelkamp BA. 2019. Identification of Novel *Acinetobacter baumannii* Host Fatty Acid Stress Adaptation Strategies. *mBio* 10.
  27. Sun D, Crowell SA, Harding CM, De Silva PM, Harrison A, Fernando DM, Mason KM, Santana E, Loewen PC, Kumar A, Liu Y. 2016. KatG and KatE confer *Acinetobacter* resistance to hydrogen peroxide but sensitize bacteria to killing by phagocytic respiratory burst. *Life Sci* 148:31–40.
  28. Pimentel C, Le C, Tuttobene MR, Subils T, Martinez J, Sieira R, Papp-Wallace KM, Keppetipola N, Bonomo RA, Actis LA, Tolmasky ME, Ramirez MS. 2021. Human Pleural Fluid and Human Serum Albumin Modulate the Behavior of a Hypervirulent and Multidrug-Resistant (MDR) *Acinetobacter baumannii* Representative Strain. *Pathogens* 10:471.
  29. Cha C, Gao P, Chen YC, Shaw PD, Farrand SK. 1998. Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria. *Mol Plant Microbe Interact* 11:1119–29.
  30. Lomovskaya O, Nelson K, Rubio-Aparicio D, Tsivkovski R, Sun D, Dudley MN. 2020. The Impact of Intrinsic Resistance Mechanisms on Potency of QPX7728, a New Ultra-Broad-Spectrum Beta-lactamase Inhibitor of Serine and Metallo Beta-Lactamases in Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. *Antimicrob Agents Chemother*.
  31. Ray A, Perez F, Beltramini AM, Jakubowycz M, Dimick P, Jacobs MR, Roman K, Bonomo RA, Salata RA. 2010. Use of vaporized hydrogen peroxide decontamination during an outbreak of multidrug-resistant *Acinetobacter baumannii* infection at a long-term acute care hospital. *Infect Control Hosp Epidemiol* 31:1236–41.

32. Chmielarczyk A, Higgins PG, Wojkowska-Mach J, Synowiec E, Zander E, Romaniszyn D, Gosiewski T, Seifert H, Heczko P, Bulanda M. 2012. Control of an outbreak of *Acinetobacter baumannii* infections using vaporized hydrogen peroxide. *J Hosp Infect* 81:239–45.
33. Martinez J, Fernandez JS, Liu C, Hoard A, Mendoza A, Nakanouchi J, Rodman N, Courville R, Tuttobene MR, Lopez C, Gonzalez LJ, Shahrestani P, Papp-Wallace KM, Vila AJ, Tolmasky ME, Bonomo RA, Sieira R, Ramirez MS. 2019. Human pleural fluid triggers global changes in the transcriptional landscape of *Acinetobacter baumannii* as an adaptive response to stress. *Sci Rep* 9:17251.
34. Jacobs AC, Thompson MG, Black CC, Kessler JL, Clark LP, McQueary CN, Gancz HY, Corey BW, Moon JK, Si Y, Owen MT, Hallock JD, Kwak YI, Summers A, Li CZ, Rasko DA, Penwell WF, Honnold CL, Wise MC, Waterman PE, Lesho EP, Stewart RL, Actis LA, Palys TJ, Craft DW, Zurawski DV. 2014. AB5075, a Highly Virulent Isolate of *Acinetobacter baumannii*, as a Model Strain for the Evaluation of Pathogenesis and Antimicrobial Treatments. *MBio* 5:e01076-14.
35. Gallagher LA, Ramage E, Weiss EJ, Radey M, Hayden HS, Held KG, Huse HK, Zurawski DV, Brittnacher MJ, Manoil C. 2015. Resources for Genetic and Genomic Analysis of Emerging Pathogen *Acinetobacter baumannii*. *J Bacteriol* 197:2027–35.
36. Rodman Nyah MJ, 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. 2019. Human Pleural Fluid Elicits Pyruvate and Phenylalanine Metabolism in *Acinetobacter baumannii* to Enhance Cytotoxicity and Immune Evasion. *Frontiers in Microbiology* 10:1581.
37. Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841–2.
38. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* 25:402–8.
39. Sartorio MG, Repizo GD, Cortez N. 2020. Catalases of the polyextremophylic Andean isolate *Acinetobacter* sp. Ver 3 confer adaptive response to H₂O₂. *FEBS J* 287:4525–4539.
40. Paulk Tierney AR, Rather PN. 2019. Methods for Detecting N-Acyl Homoserine Lactone Production in *Acinetobacter baumannii*. *Methods Mol Biol* 1946:253-258.

Figures

Figure 1

A) Venn diagram shown the transcriptional analysis comparison between *A. baumannii* AB5075 vs *A. baumannii* Δhns (both strains cultured in LB broth) and AB5075 (cultured in LB broth) vs *A. baumannii* AB5075 (cultured in LB broth supplemented with 0.2 % HSA). B) Heat-map shown the total of 11 DEGs (hhDGEs) found in common between the two analyses. C) qRT-PCR analysis of *hns* of *A. baumannii* AB5075 cultured in LB broth vs AB5075 cultured in LB broth supplemented with 3.5 % HAS. Fold changes

were calculated using double ΔC_t analysis. At least three independent samples were used, and four technical replicates were performed from each sample. Student's *t* test analysis was performed using GraphPad Prism (GraphPad software, San Diego, CA, USA). A *P* < 0.05 was considered significant.

Figure 2

Genetic analysis of genes of *A. baumannii* AB5075 or AB5075 pMBLe-*hns* cultured in LB broth or LB broth supplemented with 3.5 % HSA. qRT-PCR of genes associated with quorum sensing, *acdA*, *kar*, and *fadD*, with quorum quenching, *aidA*, with biofilm, *csuAB*, with β -oxidation, *fadA*, with stress, *katE* and *ywrO* and metabolism *phaC* expressed in LB or LB supplemented with HSA. Fold changes were calculated using double ΔC_t analysis. At least three independent samples were used, and four technical replicates were performed from each sample. The *A. baumannii* AB5075 cultured in LB was used as reference. Statistical significance ($P < 0.05$) was determined by ANOVA followed by Tukey's multiple-comparison test, one asterisks: $P < 0.05$; two asterisks: $P < 0.01$ and three asterisks: $P < 0.001$. This figure was performed using GraphPad Prism version number 9 (GraphPad software, San Diego, CA, USA, <https://www.graphpad.com/>).

A

B

Figure 3

Phenotypic analysis of biofilm formation. Biofilm assays performed with the AB5075 strain grown in LB or LB supplemented with 3.5 % HSA, Δhns and Δhns pMBLe-*hns* strains cultured in LB, and AB5075 pMBLe-*hns* culture in LB or LB supplemented with 3.5 % HSA. A) Quantification of the ratio of biofilm to total biomass. The mean $\pm$ SD is informed of three independent experiments. Statistical significance ($P < 0.05$) was determined by ANOVA followed by Tukey's multiple comparison test, one asterisks: $P < 0.05$;

two asterisks: $P < 0.01$ and three asterisks: $P < 0.001$. B) Tubes of biofilm experiment after the stained with 1% crystal violet y removed the excess. A representative experiment is shown. This figure was performed using GraphPad Prism version number 9 (GraphPad software, San Diego, CA, USA, <https://www.graphpad.com/>).

Figure 4

Phenotypic analysis of catalase activity. Monitoring the decrease in absorbance at 240 nm of crude extract of *A. baumannii* AB5075 and derivate strains cultured in LB or LB supplemented with 3.5 % HSA. The mean $\pm$ SD is informed of three independent experiments. Statistical significance ($P < 0.05$) was determined by ANOVA followed by Tukey's multiple comparison test, one asterisks: $P < 0.05$; two asterisks: $P < 0.01$ and three asterisks: $P < 0.001$. This figure was performed using GraphPad Prism version number 9 (GraphPad software, San Diego, CA, USA, <https://www.graphpad.com/>).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)