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Human serum albumin-induced modification of Ton-B-dependent receptor expression in cefiderocol-exposed carbapenem-resistant *Acinetobacter baumannii*

Editor: Prof. Q. Yang

Dear Editor,

Acinetobacter baumannii is a nosocomial pathogen that persists in the hospital environment and is resistant to multiple antimicrobial agents, and therefore is of significant clinical concern. *A. baumannii* is the causative agent of difficult-to-treat infections that are resistant to most currently available antibiotics and are associated with increased mortality. Such infections are a major problem in hospitals, particularly in intensive care units.

Human serum albumin (HSA) induces numerous phenotypic changes in *A. baumannii*, the most remarkable effects of which include modifications in transformation frequency, pathogenesis, survival, and antibiotic susceptibility levels [1,2]. Of particular interest, the presence of HSA is associated with an increase in the minimum inhibitory concentration (MIC) of cefiderocol (CFDC), one of the few therapeutic options for carbapenem-resistant *A. baumannii* (CRAB) infections. In addition, *A. baumannii* cells cultured in HSA-depleted human fluids express higher levels of the TonB-dependent transporters (TBDRs) coded for by *piuA, pirA, bauA*, and *bfnH*. TBDRs are critical for iron acquisition under iron-limiting conditions. Considering these observations, the HSA-mediated effects may be due to the iron associated with this host protein. This hypothesis is supported by results indicating that the expression of TBDRs and the MIC of CFDC are not modified in the presence of iron-depleted HSA [3]. Hence, HSA might act as an iron source.

HSA and CFDC bind ferric iron; therefore, the effects of these components alone and in combination on the expression of TBDR genes in CRAB strains were studied. Assays were conducted using physiological concentrations of HSA and subinhibitory concentrations of CFDC. *A. baumannii* strains used were the model strain, AB5075 ($bla_{\text{OXA-51-like}}$, bla_{ADC}, $bla_{\text{OXA-23}}$, $bla_{\text{GES-11}}$), and the CRAB clinical isolates, AMA40 ($bla_{\text{OXA-51-like}}$,bla_{ADC}, $bla_{\text{NDM-1}}$), AMA16 ($bla_{\text{OXA-51-like}}$, $bla_{\text{NDM-1}}$, $bla_{\text{PER-7}}$, ISA*ba125*), and AB0057 ($bla_{\text{OXA-51-like}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-23}}$, bla_{ADC}) [4–6] (Table S1). All four *A. baumannii* strains were cultured in iron-depleted cation adjusted Mueller Hinton (CAMHB) (Becton Dickinson, USA) or CAMHB supplemented with 1) a sub-MIC of CFDC, b) 3.5% HSA (Sigma-Aldrich, USA), or c) two sub-MIC of CFDC plus 3.5% HSA. After culturing, total RNA was extracted and used in quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays.

Transcriptional expression levels of *pirA, piuA, bauA*, and *bfnH* in cells cultured in CAMHB plus HSA were significantly lower than those in cells of all four strains cultured in plain CAMHB (Fig. 1). In contrast, expression levels of these genes were higher in cells cultured in the presence of CFDC (Fig. 1). Notably, the levels of expression of these genes were lower when cells were cultured in medium supplemented with both HSA and CFDC (Fig. 1 and Table S2). Table S2 shows the $\log_2$-fold change for all the genes that were repressed (in red, negative values) or induced (in green, positive values) under the aforementioned experimental conditions. The reduced expression of bacterial genes involved in capturing iron by high-affinity iron uptake systems may be due to the contribution of ferric iron provided by HSA. This condition results in an iron-abundant milieu in which high-affinity iron-uptake coding genes involved in CFDC uptake are turned off. The experimental data shown in Fig. 1 also indicate that adding CFDC to the culture medium enhances transcription, possibly because it acts as an iron chelator. Such activity is significantly diminished when both CFDC and HSA are present in the medium. Taken together, these results could explain the reduced susceptibility to CFDC when HSA is present in the extracellular medium, even in the presence of CFDC. Thus, human fluids containing naturally iron-rich HSA negatively impact the therapeutic action of CFDC. Future studies are needed to confirm the role of iron and HSA on the expression of genes required for CFDC biological activity, and to shed light on the molecular mechanism by which the HSA-provided ferric iron reaches the bacterial cytosol.

As CFDC is a β-lactam, modifications in the expression of β-lactamase genes could have an additional effect on susceptibility. Analysis of the impact of HSA and CFDC on expression levels of β-lactamase genes failed to show a clear pattern (Fig. S1). There were wide variations in β-lactamase gene transcriptional responses, which could in part be because the strains tested in this study belong to different clonal complexes (Table S1). The current study results indicate that the changes in resistance are not associated with an increase in β-lactamase production; however, more studies on a larger number of strains and a broader spectrum of β-lactamase genes are needed.

In a further evaluation, CFDC MICs for the four CRAB strains were tested in the presence of 25 μM $FeCl_2$. There were no significant differences in MIC between the absence or presence of $FeCl_2$ (Table S3). These results agree with the observation that CFDC interacts with ferric ion.

Lastly, to expand the previous observations, the CFDC MICs of AMA16 and AB0057 were determined in the presence of either HSA or iron-free HSA. A 2-fold increase in MIC was observed for AB0057 in the presence of HSA, whereas MIC decreased to basal levels in the presence of iron-free HSA (Table S4). AMA16 MIC was >256 mg/L in all tested conditions (Table S4). These results agree with our recently published research on the CRAB AB5075 and AMA40 strains in which the importance of ferric HSA on CFDC MICs was shown [3].

In summary, the effectiveness of CFDC against *A. baumannii* is reduced regardless of the presence of CFDC in iron-rich HSA-containing fluids. Investigations into the underlying mechanisms of this reduction are needed to develop strate-

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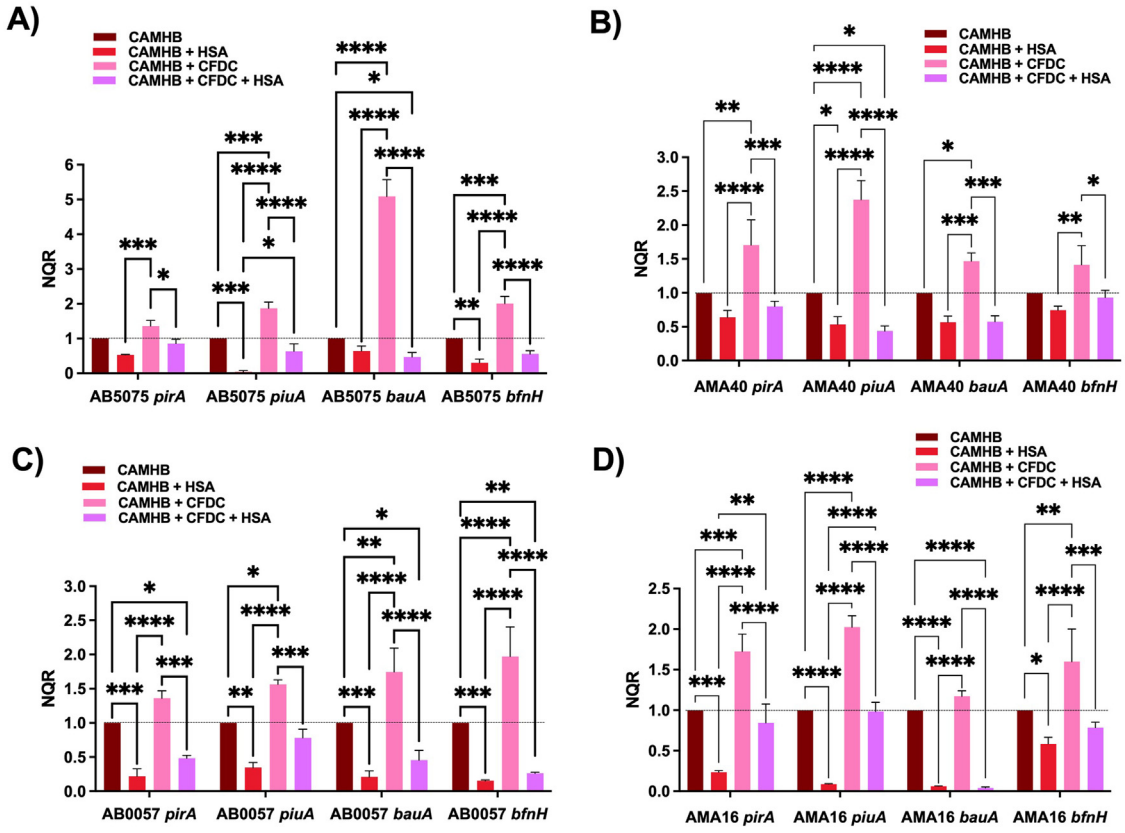

Figure 1. Expression analysis of iron uptake genes in the AB5075 (A), AMA40 (B), AB0057 (C) and AMA 16 (D) strains. qRT-PCR of TonB-dependent receptors (*pirA, piuA, bauA, and bfnH*) expressed in the presence of human serum albumin (HSA), cefiderocol (CFDC), or HSA + CFDC. The data presented are the mean ± standard deviation (SD) of normalized relative quantities (NRQ) derived from transcript levels calculated using the qBASE method. The qBASE method is a modification of the ΔΔCt method that considers multiple reference genes (*rpoB* and *recA*) and gene-specific amplification efficiencies. Three independent samples were used, and three technical replicates were performed from each sample. The reference condition used in the analysis was unsupplemented CAMHB. Statistical significance was determined using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Asterisks indicate the significance levels for the statistical analysis: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$.

gies that counteract the negative impact of host ferric-HSA and enhance the therapeutic potential of CFDC against this challenging pathogen.

Declarations

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Ethical Approval: Not required

Sequence Information: Not applicable

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2023.106950.

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