

**Table S1:** Comparison of the level of expression of iron associated and antibiotic resistance genes obtained by qRT-PCR in the two CRAB strains

Gene name	AB5075 (Log <sub>2</sub> FC)		AMA40 (Log <sub>2</sub> FC)	
	HS HSA free	HPF HSA free	HS HSA free	HPF HSA free
TonB-dependent Receptors (TBDRs)				
<i>pirA</i>	3.36	2.68	2.75	1.82
<i>piuA</i>	3.92	3.70	2.29	2.75
<i>bauA</i>	2.18	3.44	2.65	1.50
<i>bfnH</i>	2.37	2.92	3.43	2.43
β-lactamases genes				
<i>bla</i> <sub>OXA-51</sub>	-1.38	-0.84	-0.82	-0.88
<i>bla</i> <sub>ADC</sub>	-0.16	-1.14	-1.47	-1
<i>bla</i> <sub>OXA-23</sub>	-0.09	-0.89		
<i>bla</i> <sub>GES-14</sub>	0.08	-1.05		
<i>bla</i> <sub>NDM-1</sub>			-1.25	-0.44

- Log<sub>2</sub>FC > 1 (p<0.05)     ● Log<sub>2</sub>FC < - 1 (p<0.05)  
● Log<sub>2</sub>FC (0-1)     ● Log<sub>2</sub>FC (-1-0)

**Table S2.** CFDC MICs for two CRAB representative strains performed using CFDC MTS strips (Liofilchem S.r.l., Italy) on Iron-depleted CAMHA (Cation Adjusted Mueller Hinton Agar) supplemented with 20  $\mu$ M or 40  $\mu$ M FeCl<sub>3</sub>.

Strain	CAMHA	CAMHA + 20 $\mu$ M FeCl <sub>3</sub>	CAMHA + 40 $\mu$ M FeCl <sub>3</sub>
AMA40	0.25	1.5	3.0
AB5075	0.19	1.5	2.0

CAMHA: Iron-depleted Cation Adjusted Mueller Hinton (treated with CHELEX as recommend by the CLSI).

**Table S3:** Minimal Inhibitory Concentrations (MICs) of cefiderocol (CFDC) for the CRAB AB5075 and AMA40 strains, performed using CFDC MTS strips (Liofilchem S.r.l., Italy) on Iron-depleted CAMHA (Cation Adjusted Mueller Hinton Agar) and supplemented with 20% of cerebrospinal fluid (CSF).

Condition	CFDC MIC ( <i>mg/L</i> )	
	AB5075	AMA40
CAMHB	0.50 (S)	0.50 (S)
20% CSF	0.38 (S)	0.75 (S)

CFDC: cefiderocol, S: Susceptible, R: Resistant

**Table S4.** Name of the primers used in the present study and their corresponding sequences.

Primer Name	Function	5'-3' Sequence
<i>bla</i> <sub>OXA-51-like</sub> F	$\beta$ -lactamase	ATGAACATTAAAACACTCTT
<i>bla</i> <sub>OXA-51-like</sub> R	$\beta$ -lactamase	TATAAAATACCTAATTGTTC
<i>bla</i> <sub>OXA-23</sub> F	$\beta$ -lactamase	CGAGTCAGATTGTTCAAGGA
<i>bla</i> <sub>OXA-23</sub> R	$\beta$ -lactamase	GCTCAACCCAGCCGGTCAAC
<i>bla</i> <sub>GES-14</sub> F	$\beta$ -lactamase	ACGCACTATTACTGGCAGGG
<i>bla</i> <sub>GES-14</sub> R	$\beta$ -lactamase	TCTCCTTGGGGATCGACGAT
<i>bla</i> <sub>NDM-1</sub> F	$\beta$ -lactamase	GAAGCTGAGCACCGCATTAG
<i>bla</i> <sub>NDM-1</sub> R	$\beta$ -lactamase	AACCAGATCGCCAAACCGTT
<i>bauA</i> F	Iron uptake	AAATGTTTGGCCGCGTTGAGGT
<i>bauA</i> R	Iron uptake	CAATCGTGCAAACGGTTCATCAGC
<i>pirA</i> F	Iron uptake	GTCTATGGCTTTTGCTGCACA
<i>pirA</i> R	Iron uptake	GCGATTGCTTCACTTGCTCT
<i>piuA</i> F	Iron uptake	ATGGCGCAAGAAGCAGTTTC
<i>piuA</i> R	Iron uptake	TGTCTTTGAGAGGAGCCACG
<i>bfnH</i> F	Iron uptake	ACTGCGACTCGTACACCAAA
<i>bfnH</i> R	Iron uptake	ACTTTACGACCTGCCGTAGC
<i>bla</i> <sub>ADC</sub> F ( <i>ampC</i> )	$\beta$ -lactamase	TGCCAACCTTAACCCACA
<i>bla</i> <sub>ADC</sub> R ( <i>ampC</i> )	$\beta$ -lactamase	AGTTTGTAACGTTGCCGG

**Table S5.** Total protein concentration determined in CAMHB and different fluids analyzed using the ProteoExtract® Albumin/IgG Removal Kit (Sigma-Aldrich, MA, United States) following the manufacturer's recommendations.

	Protein concentration (mg/mL)	Reference
CAMHB	BLQ	This work
HPF	22.66 ± 4.53	This work
HPF HSA Free	BLQ	This work
HS	14.15 ± 0.31	This work
HS HSA Free	BLQ	This work

CAMHB: iron depleted cation adjusted Mueller Hinton, HSA: human serum albumin, HPF: human pleural fluids, HS: human serum, BLQ: below limit of quantification.

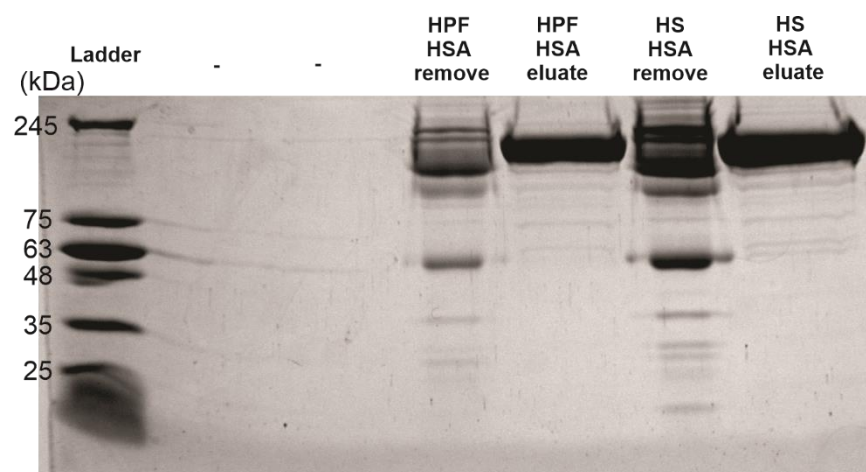
**Table S6.** Total Iron concentration determined in CAMHB and different fluids analyzed using the Iron Assay Kit (Sigma-Aldrich, MA, United States) following the manufacturer's recommendations.

	[Fe] total ( $\mu\text{M}$ )	Reference
CAMHB	BLQ	This work
HSA	$65.4 \pm 0.39$	This work
HSA Fe Free	BLQ	This work
HPF	$129.97 \pm 0.86$	[1]
HPF HSA Free	BLQ	This work
HS	$65.45 \pm 0.46$	This work
HS HSA Free	BLQ	This work
CSF	BLQ	This work

CAMHB: iron depleted cation adjusted Mueller Hinton, HSA: human serum albumin, HPF: human pleural fluids, HS: human serum, CSF: cerebrospinal fluid, BLQ: below limit of quantification.

- [1] C. Pimentel *et al.*, "Human pleural fluid and human serum albumin modulate the behavior of a hypervirulent and multidrug-resistant (MDR) acinetobacter Baumannii representative strain," *Pathogens*, vol. 10, no. 4, pp. 1–13, 2021, doi: 10.3390/pathogens10040471.

**Figure S1.** SDS-PAGE of human fluids analyzed using the ProteoExtract® Albumin/IgG Removal Kit (Sigma-Aldrich, MA, United States)



HSA: human serum albumin, HPF: human pleural fluids, HS: human serum.