Impact of Human Serum Proteins on Susceptibility of *Acinetobacter baumannii* to Cefiderocol: role of iron transport

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Abstract

Cefiderocol is a siderophore antibiotic that co-opts iron transporters to facilitate cell entry. We show that genes related to iron uptake systems and resistance to β -lactams in *Acinetobacter baumannii* have altered expression levels in the presence of human serum, human serum albumin, or human pleural fluid. Cefiderocol MICs are also raised in the presence of the mentioned fluids. Clinical response in *A. baumannii* infections may be related to the interplay of these human factors.

Carbapenem-resistant *Acinetobacter baumannii* (CRAB), one of the most feared pathogens in healthcare settings, driving the Centers for Disease Control and Prevention (CDC) to categorize it as an “urgent threat” (1-5). As a consequence of *A. baumannii*’s capability to develop multidrug resistance (MDR), treatment strategies become extremely limited, with only a few active antibiotics (6-8).

The increasing number of CRAB infections translates into alarmingly high morbidity and mortality (2, 9-11). Consequently, numerous efforts are focusing on finding novel treatment options (12-19). Cefiderocol (CFDC), formerly known as S-649266, approved by the FDA in November 2019 to treat nosocomial pneumonia and urinary tract infections, is a hybrid molecule that consists of a cephalosporin component that targets cell wall synthesis and a catechol siderophore moiety that allows cell penetration by active ferric-siderophore transporters (20-24). This novel synthetic compound uses a “Trojan horse” strategy to improve antibiotic penetration and reach a high concentration at the target site (21, 22). CFDC’s primary targets are cell wall synthesizing proteins associated with β -lactam activity (PBP1 and PBP3) (25). Early research showed promising results when treating Gram-negative carbapenem-resistant infections (7, 26, 27). Recently, a study described a number of unfavorable outcomes in patients with pulmonary or bloodstream *A. baumannii* infections compared to other available therapies (7). Our goal was to determine if there is a molecular basis for this observation.

A. baumannii senses components of human fluids and responds by modifying its transcriptional and phenotypic profiles (28-32). Human serum albumin (HSA) as well as human pleural fluid (HPF) modulate the expression of

genes associated with iron-uptake systems, biofilm formation, antibiotic resistance, and DNA-acquisition among others (29, 33, 34). In previous studies we observed that CRAB AB5075 genes associated with iron uptake systems were down-regulated when exposed to HPF and 0.2% HSA (29), while genes associated with β -lactam resistance were up-regulated in the presence of physiological concentrations of HSA and human serum (HS) (33, 34). The modification in expression of iron-uptake systems and β -lactam resistance genes could be responsible for variations in *A. baumannii* susceptibility to CFDC, and concomitantly for the unfavorable outcomes of patients with pulmonary or bloodstream infections. In this work we describe the effect of HS, HSA, and HPF on representative CRAB strains.

CRAB AB0057 and AMA16 are clinical strains that belong to different clonal complexes and harbor OXA-23 and NDM-1, respectively (35, 36). Quantitative RT-PCR (qRT-PCR) assays were carried out using total RNA extracted from cells cultured in lysogeny broth (LB) or LB supplemented with 4% HPF or 3.5% HSA, or cultured in HS (Fig. 1A and B), as previously described (29).

The expression of iron uptake related genes *bauA*, *basE*, *feoA* and *tonB*, was reduced in both strains under most of the conditions evaluated (Fig. 1 A and B). In addition, when the hypervirulent *A. baumannii* AB5075 strain was exposed to 3.5% HSA or HS, the expression levels of most genes analyzed were down regulated. Pimentel *et al.* previously showed that levels of expression of AB5075 iron related genes were down-regulated in cells cultured in iron-rich media (29); this finding is in agreement with level of expression of *basE* reported in this work (Fig. 1C). The *fhuE_1*, *fhuE_2*, *pirA* and *piuA* genes,

which are associated with iron-uptake (37), were also down-regulated in the presence of HPF (Fig. S1 A). Moreover, the transcriptional levels of these genes were further analyzed by qRT-PCR for the three strains (AMA16, AB0057, and AB5075) in the three tested conditions (HFP, HS, and HSA). In most of conditions evaluated, levels of expression were down-regulated (Fig. S1 B).

Genes associated with β -lactam resistance, such as *bla*_{OXA-51-like}, *bla*_{OXA-23}, *pbp1* and *pbp3* (33, 34), were up-regulated in *A. baumannii* AB5075, AMA16, and AB0057 when cultured in the presence of 3.5% HSA. To further examine if changes in genes associated with β -lactam resistance are affected by HS or HPF in the CRAB strains, qRT-PCR was used to assess gene expression of *bla*_{OXA-51-like}, *bla*_{OXA-23}, *bla*_{NDM-1}, *pbp1*, *pbp3*, and *carO* in cultures of strains AMA16 and AB0057 containing HS or HPF. This transcriptional analysis revealed a down-regulation in expression levels of *bla*_{NDM-1} in *A. baumannii* AMA16 cultured in the presence of HS or HPF (Fig. 2 A). In this strain, the levels of expression of *ISAb_a125*, *bla*_{PER-7}, *pbp1*, and *pbp3* were up-regulated in the presence of HPF. HS also induced up-regulation of *ISAb_a125* (Fig. 2 A). In the case of the AB0057 strain, we observed up-regulation of *bla*_{OXA-23}, *bla*_{OXA-51}, *carO*, *bla*_{ADC}, *pbp1* and *pbp3* in cultures containing HPF. In cultures containing HS the former four genes were up-regulated (Fig. 2 B). Assessment of the changes in β -lactam resistance-associated *A. baumannii* AB5075 genes in the presence of HPF showed down-regulation of the *carO* and *bla*_{GES-14} genes (Fig. 2 C). On the other hand, the expression levels of *bla*_{OXA-51}, *pbp1*, *pbp3* and *bla*_{ADC} were increased (Fig. 2 C).

The impact of human fluids in CFDC activity was further studied using a panel of twenty CRAB strains including AB5075, AMA16, and AB0057 (a strain isolated in the Walter Reed Army Medical Center (38). Cells cultured in LB, and LB supplemented with HPF or 3.5 % HSA were used to determine the MICs of CFDC. Ten strains had baseline resistance to CFDC (Table S1). An increase in the MIC values was observed in 14 strains when the cells were growing in the presence of HSA or HPF (Table S1 and Fig. 2 D). We draw attention to the occurrence of heteroresistant colonies with the inhibition ellipse in 10 of the tested strains (Fig. S2). Most of the strains that exhibited this phenomenon harbored *bla*_{PER-7} (35). Our results agree with a recent report indicating that PER-like β -lactamases contribute to a decreased susceptibility to CFDC in *A. baumannii* (39). In addition, they observed that the combination of CFDC with avibactam may inhibit the activity of PER-type β -lactamases (39).

Overall, our results showed that *A. baumannii* responds to the interaction with human bodily fluids during the establishment of infection modulating the expression of iron-uptake genes and β -lactam associated genes. We hypothesize that the presence of iron binding proteins in the human fluids is sensed by *A. baumannii*, which in turn responds by down-regulating expression of iron uptake system genes impacting CFDC activity. The particular changes in gene expression of the aforementioned group of genes, which is not evaluated in traditional susceptibility tests, may contribute to unfavorable outcomes associated with iron-rich environments. These results also raise questions regarding the expression of other factors that may contribute to challenges in overcoming infections by *A. baumannii*.

The limitations inherent to performing MICs in the presence of bodily fluids (protein binding) is recognized. We are also aware that comparing these analysis does not take into account pharmacokinetics and pharmacodynamics (PK/PD) parameters that usure drug efficacy (40, 41). Laslty, we appreciate that these data must also be evaluated in the context of outcomes of animal models of infection (42-44). Nevertheless, the impact on iron transport mechanisms uncovered in these observations merits further analyses.

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Supplementary Materials

Table S1: Minimal Inhibitory Concentrations of Cefiderocol (CFDC) for 22 Carbapenem-resistant *Acinetobacter baumannii* representative strains performed using CFDC MTS strips ((Liofilchem S.r.l., Italy) on Mueller Hinton Agar (cation adjusted). *A. baumannii* cells were cultured in LB or LB supplemented with 3.5 % HSA or HPF, respectively.

Figure S1: Differential expression of *fhuE_1*, *fhuE_2*, *pirA* and *piuA* genes associated with iron-uptake obtained for *A. baumannii* AB5075 cultured in the presence of HSA and HPF.

Figure S2: Intra-colonies in *A. baumannii* AMA40 representative of the tested strains in the presence of HPF.

References

1. Eichenberger EM, Thaden JT. 2019. Epidemiology and Mechanisms of Resistance of Extensively Drug Resistant Gram-Negative Bacteria. Antibiotics (Basel) 8.
2. Holmes CL, Anderson MT, Mobley HLT, Bachman MA. 2021. Pathogenesis of Gram-negative bacteremia. Clin Microbiol Rev 34.
3. Garcia-Ortega L, Arch O, Perez-Canosa C, Lupion C, Gonzalez C, Rodriguez-Bano J, Spanish Study Group of Nosocomial I. 2011. Control measures for *Acinetobacter baumannii*: a survey of Spanish hospitals. Enferm Infecc Microbiol Clin 29:36-8.
4. Ramirez MS, Bonomo RA, Tolmasky ME. 2020. Carbapenemases: Transforming *Acinetobacter baumannii* into a Yet More Dangerous Menace. Biomolecules 10.
5. CDC. 2019. Antibiotic Resistance Threats in the United States. Atlanta, GA: US Department of Health and Human Services, CDC; 2019.
6. Karaikos I, Lagou S, Pontikis K, Rapti V, Poulakou G. 2019. The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. Front Public Health 7:151.
7. Bassetti M, Echols R, Matsunaga Y, Ariyasu M, Doi Y, Ferrer R, Lodise TP, Naas T, Niki Y, Paterson DL, Portsmouth S, Torre-Cisneros J, Toyozumi K, Wunderink RG, Nagata TD. 2021. Efficacy and safety of cefiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria (CREDIBLE-CR): a randomised, open-label, multicentre, pathogen-focused, descriptive, phase 3 trial. Lancet Infect Dis 21:226-240.
8. Bassetti M, Peghin M, Vena A, Giacobbe DR. 2019. Treatment of Infections Due to MDR Gram-Negative Bacteria. Front Med (Lausanne) 6:74.
9. Isler B, Doi Y, Bonomo RA, Paterson DL. 2019. New treatment options against carbapenem-resistant *Acinetobacter baumannii* infections. Antimicrob Agents Chemother 63.
10. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF. 2018. Carbapenemase-Producing Organisms: A Global Scourge. Clin Infect Dis 66:1290-1297.
11. Spellberg B, Bonomo RA. 2014. The deadly impact of extreme drug resistance in *Acinetobacter baumannii*. Crit Care Med 42:1289-91.
12. Paterson DL, Isler B, Stewart A. 2020. New treatment options for multiresistant gram negatives. Curr Opin Infect Dis 33:214-223.
13. McLeod SM, Moussa SH, Hackel MA, Miller AA. 2020. In Vitro Activity of Sulbactam-Durlobactam against *Acinetobacter baumannii-calcoaceticus* Complex Isolates Collected Globally in 2016 and 2017. Antimicrob Agents Chemother 64.

14. 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.
15. Karaiskos I, Galani I, Souli M, Giamarellou H. 2019. Novel beta-lactam-beta-lactamase inhibitor combinations: expectations for the treatment of carbapenem-resistant Gram-negative pathogens. Expert Opin Drug Metab Toxicol 15:133-149.
16. Barnes MD, Kumar V, Bethel CR, Moussa SH, O'Donnell J, Rutter JD, Good CE, Hujer KM, Hujer AM, Marshall SH, Kreiswirth BN, Richter SS, Rather PN, Jacobs MR, Papp-Wallace KM, van den Akker F, Bonomo RA. 2019. Targeting Multidrug-Resistant *Acinetobacter* spp.: Sulbactam and the Diazabicyclooctenone beta-Lactamase Inhibitor ETX2514 as a Novel Therapeutic Agent. mBio 10.
17. Zhanel GG, Lawrence CK, Adam H, Schweizer F, Zelenitsky S, Zhanel M, Lagace-Wiens PRS, Walkty A, Denisuk A, Golden A, Gin AS, Hoban DJ, Lynch JP, 3rd, Karlowsky JA. 2018. Imipenem-Relebactam and Meropenem-Vaborbactam: Two Novel Carbapenem-beta-Lactamase Inhibitor Combinations. Drugs 78:65-98.
18. Wright H, Bonomo RA, Paterson DL. 2017. New agents for the treatment of infections with Gram-negative bacteria: restoring the miracle or false dawn? Clin Microbiol Infect 23:704-712.
19. Durand-Reville TF, Guler S, Comita-Prevoir J, Chen B, Bifulco N, Huynh H, Lahiri S, Shapiro AB, McLeod SM, Carter NM, Moussa SH, Velez-Vega C, Olivier NB, McLaughlin R, Gao N, Thresher J, Palmer T, Andrews B, Giacobbe RA, Newman JV, Ehmann DE, de Jonge B, O'Donnell J, Mueller JP, Tommasi RA, Miller AA. 2017. ETX2514 is a broad-spectrum beta-lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*. Nat Microbiol 2:17104.
20. Parsels KA, Mastro KA, Steele JM, Thomas SJ, Kufel WD. 2021. Cefiderocol: a novel siderophore cephalosporin for multidrug-resistant Gram-negative bacterial infections. J Antimicrob Chemother 76:1379-1391.
21. Bonomo RA. 2019. Cefiderocol: A Novel Siderophore Cephalosporin Defeating Carbapenem-resistant Pathogens. Clin Infect Dis 69:S519-S520.
22. Zhanel GG, Golden AR, Zelenitsky S, Wiebe K, Lawrence CK, Adam HJ, Idowu T, Domalaon R, Schweizer F, Zhanel MA, Lagace-Wiens PRS, Walkty AJ, Noreddin A, Lynch JI JP, Karlowsky JA. 2019. Cefiderocol: A Siderophore Cephalosporin with Activity Against Carbapenem-Resistant and Multidrug-Resistant Gram-Negative Bacilli. Drugs 79:271-289.
23. Aoki T, Yoshizawa H, Yamawaki K, Yokoo K, Sato J, Hisakawa S, Hasegawa Y, Kusano H, Sano M, Sugimoto H, Nishitani Y, Sato T, Tsuji M, Nakamura R, Nishikawa T, Yamano Y. 2018. Cefiderocol (S-649266), A new siderophore cephalosporin exhibiting potent activities against *Pseudomonas aeruginosa* and other gram-negative pathogens including

- multi-drug resistant bacteria: Structure activity relationship. Eur J Med Chem 155:847-868.
24. Ito A, Nishikawa T, Matsumoto S, Yoshizawa H, Sato T, Nakamura R, Tsuji M, Yamano Y. 2016. Siderophore Cephalosporin Cefiderocol Utilizes Ferric Iron Transporter Systems for Antibacterial Activity against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 60:7396-7401.
25. Sato T, Yamawaki K. 2019. Cefiderocol: Discovery, Chemistry, and In Vivo Profiles of a Novel Siderophore Cephalosporin. Clin Infect Dis 69:S538-S543.
26. Wunderink RG, Matsunaga Y, Ariyasu M, Clevenbergh P, Echols R, Kaye KS, Kollef M, Menon A, Pogue JM, Shorr AF, Timsit JF, Zeitlinger M, Nagata TD. 2021. Cefiderocol versus high-dose, extended-infusion meropenem for the treatment of Gram-negative nosocomial pneumonia (APEKS-NP): a randomised, double-blind, phase 3, non-inferiority trial. Lancet Infect Dis 21:213-225.
27. Jacobs MR, Abdelhamed AM, Good CE, Rhoads DD, Hujer KM, Hujer AM, Domitrovic TN, Rudin SD, Richter SS, van Duin D, Kreiswirth BN, Greco C, Fouts DE, Bonomo RA. 2019. ARGONAUT-I: Activity of Cefiderocol (S-649266), a Siderophore Cephalosporin, against Gram-Negative Bacteria, Including Carbapenem-Resistant Nonfermenters and *Enterobacteriaceae* with Defined Extended-Spectrum beta-Lactamases and Carbapenemases. Antimicrob Agents Chemother 63.
28. Martinez J, Razo-Gutierrez C, Le C, Courville R, Pimentel C, Liu C, Fung SE, Tuttobene MR, Phan K, Vila AJ, Shahrestani P, Jimenez V, Tolmasky ME, Becka SA, Papp-Wallace KM, Bonomo RA, Soler-Bistue A, Sieira R, Ramirez MS. 2021. Cerebrospinal fluid (CSF) augments metabolism and virulence expression factors in *Acinetobacter baumannii*. Sci Rep 11:4737.
29. 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.
30. 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.
31. 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.
32. 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.

33. 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.
34. Pimentel C, Le C, Tuttobene MR, Subils T, Papp-Wallace KM, Bonomo RA, Tolmasky ME, Ramirez MS. 2021. Interaction of *Acinetobacter baumannii* with Human Serum Albumin: Does the Host Determine the Outcome? Antibiotics (Basel) 10.
35. Adams MD, Pasteran F, Traglia GM, Martinez J, Huang F, Liu C, Fernandez JS, Lopez C, Gonzalez LJ, Alborno E, Corso A, Vila AJ, Bonomo RA, Ramirez MS. 2020. Distinct mechanisms of dissemination of NDM-1 metallo- beta-lactamase in *Acinetobacter* spp. in Argentina. Antimicrob Agents Chemother.
36. Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H, Jamison JJ, MacDonald IJ, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. 2008. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J Bacteriol 190:8053-8064.
37. Tiwari V, Rajeswari MR, Tiwari M. 2019. Proteomic analysis of iron-regulated membrane proteins identify FhuE receptor as a target to inhibit siderophore-mediated iron acquisition in *Acinetobacter baumannii*. Int J Biol Macromol 125:1156-1167.
38. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, Ecker DJ, Massire C, Eshoo MW, Sampath R, Thomson JM, Rather PN, Craft DW, Fishbain JT, Ewell AJ, Jacobs MR, Paterson DL, Bonomo RA. 2006. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 50:4114-23.
39. Poirel L, Sadek M, Nordmann P. 2021. Contribution of PER-type and NDM-type ss-lactamases to cefiderocol resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother: AAC0087721.
40. McCreary EK, Heil EL, Tamma PD. 2021. New Perspectives on Antimicrobial Agents: Cefiderocol. Antimicrob Agents Chemother.
41. Konig C, Both A, Rohde H, Kluge S, Frey OR, Rohr AC, Wichmann D. 2021. Cefiderocol in Critically Ill Patients with Multi-Drug Resistant Pathogens: Real-Life Data on Pharmacokinetics and Microbiological Surveillance. Antibiotics (Basel) 10.
42. Kidd JM, Abdelraouf K, Nicolau DP. 2019. Development of Neutropenic Murine Models of Iron Overload and Depletion To Study the Efficacy of Siderophore-Antibiotic Conjugates. Antimicrob Agents Chemother 64.
43. Kidd JM, Abdelraouf K, Nicolau DP. 2019. Efficacy of Humanized Cefiderocol Exposure Is Unaltered by Host Iron Overload in the Thigh Infection Model. Antimicrob Agents Chemother 64.
44. Matsumoto S, Singley CM, Hoover J, Nakamura R, Echols R, Rittenhouse S, Tsuji M, Yamano Y. 2017. Efficacy of Cefiderocol against Carbapenem-Resistant Gram-Negative Bacilli in Immunocompetent-Rat Respiratory Tract Infection Models Recreating Human Plasma Pharmacokinetics. Antimicrob Agents Chemother 61.

Figure legends

Figure 1. Genetic analysis of iron uptake genes of AMA16 (A), AB0057 (B) and AB5075 (C) *A. baumannii* strains. qRT-PCR of genes associated with iron uptake, *bauA*, *basE*, *feoA* and *tonB* expressed in LB or LB supplemented with HPF or HSA, or cultured in HS. 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 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: $p < 0.001$.

Figure 2. (A-C) Genetic analysis of β -lactamase + PBP genes of AMA16 (A), AB0057 (B) and AB5075 (C) *A. baumannii* strains. qRT-PCR of genes associated with β -lactams resistance expressed in LB, LB supplemented with HPF, or in HS. Fold changes were calculated using double ΔC_t analysis. At least three independent samples were used. LB was used as the reference condition. 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: $p < 0.001$. (D) Effect of HSA and HPF on the antimicrobial susceptibility of *A. baumannii* representatives strains AMA40 and AMA113 strains grew in LB broth, LB broth plus 3.5 % HSA, or HPF were used to performed cefiderocol (CFDC) susceptibility. Minimum inhibitory concentration (MIC) on cation adjusted Mueller Hinton agar was performed by MTS (Liofilchem S.r.l., Italy) following manufacturer's recommendations.

A **B** **C**

A **B** **C** **D**