JAC Antimicrob Resist
https://doi.org/10.1093/jacamr/dlad078

JAC-Antimicrobial Resistance

Salvage therapy with sulbactam/durlobactam against cefiderocol-resistant *Acinetobacter baumannii* in a critically ill burn patient: clinical challenges and molecular characterization

Giusy Tiseo [1], Cesira Giordano[2], Alessandro Leonildi[2], Niccolò Riccardi[1], Valentina Galfo[1], Federica Limongi[3], Manuela Nicastro[3], Simona Barnini[2] and Marco Falcone [1]*

[1]*Infectious Diseases Unit, Department of Clinical and Experimental Medicine, Azienda Ospedaliero Universitaria Pisana, University of Pisa, Pisa, Italy;* [2]*Microbiology Unit, Azienda Ospedaliero Universitaria Pisana, Pisa, Italy;* [3]*Department of Anaesthesia and Critical Care Medicine, Azienda Ospedaliero Universitaria Pisana, Pisa, Italy*

*Corresponding author. E-mail: marco.falcone@unipi.it

Received 13 April 2023; accepted 23 May 2023

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections are associated with high mortality rates. The optimal treatment regimen for CRAB has not been defined. Cefiderocol has been recently introduced in the armamentarium against CRAB but there is concern about treatment-emergent resistance. Since mortality rates in CRAB infections remain high, further antibiotic options are needed.

Methods: We report a case of severe infection by CRAB resistant to both colistin and cefiderocol treated with sulbactam/durlobactam and describe the molecular features of the strain. Susceptibility to cefiderocol was detected by disc diffusion according to EUCAST breakpoints. Susceptibility to sulbactam/durlobactam was determined by Etest according to preliminary breakpoints provided by Entasis Therapeutics. Whole Genome Sequencing (WGS) of the CRAB isolate was performed.

Results: A burn patient with ventilator-associated pneumonia by CRAB resistant to colistin and cefiderocol received sulbactam/durlobactam as compassionate use. She was alive after 30 days from the end of therapy. Complete microbiological eradication of CRAB was achieved. The isolate harboured $bla_{\text{ADC-30}}$, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$. A missense mutation in PBP3 was detected. The isolate harboured a mutation in the TonB-dependent siderophore receptor gene *piuA* that showed a frameshift mutation causing a premature stop codon (K384fs). Moreover, the *fepA* gene, which is orthologous to *pirA*, was interrupted by a transposon insertion P635-IS*Aba125* (IS*30* family).

Conclusions: Further treatment options for severe infections by CRAB resistant to all available antibiotics are urgently needed. Sulbactam/durlobactam may be a future option against MDR *A. baumannii*.

Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) represents one of the pathogens responsible for an excess of death in nosocomial settings.[1] Treatment options for infections by CRAB are limited. As one of the few agents with *in vitro* activity against CRAB, colistin has been considered the backbone therapy, despite the high risk of nephrotoxicity associated with its clinical use and the limitations of available randomized controlled trials (RCTs).[2,3] The marketing authorization of cefiderocol has raised great expectations for therapy against CRAB.[4] However, results from the Phase 3 RCT CREDIBLE-CR, comparing cefiderocol with the best available therapy, showed an unexpected increase of mortality in patients with CRAB infections.[5] Moreover, observational studies reporting the real-world experience of cefiderocol showed variable results. A retrospective study comparing cefiderocol monotherapy with colistin-containing combinations in COVID-19 patients with CRAB infections failed to demonstrate a significant association of cefiderocol with better clinical outcome, probably because of insufficient power.[6] A study comparing colistin- versus cefiderocol-containing regimens demonstrated a reduced risk of death in patients with bloodstream infection (BSI), but not in those with ventilator-associated pneumonia

(VAP) by CRAB.[7] In the same study, 8.5% of patients developed cefiderocol resistance, suggesting the need for careful monitoring of the susceptibility profiles of CRAB strains exposed to this antibiotic.[7] Resistance to cefiderocol is associated with several and complex molecular mechanisms, which are only partially understood. Sulbactam is a potent PBP inhibitor with intrinsic activity against *A. baumannii*. However, its efficacy is limited, especially in monotherapy, due to its susceptibility to cleavage by β-lactamases expressed by contemporary *A. baumannii* isolates. Durlobactam, a non-β-lactam β-lactamase inhibitor, is a potent inhibitor of class A, C and D β-lactamases that restores the activity of sulbactam. Sulbactam/durlobactam may represent a new option for the treatment of CRAB infections.

Here, we discuss a case of VAP by CRAB resistant to both cefiderocol and colistin successfully treated with sulbactam/durlobactam and describe the molecular features of the strain.

Methods

Clinical case

A young female with no significant medical history was hospitalized for a severe burn injury with involvement of 45% of the total body surface area. The patient had severe clinical conditions and was intubated. On Day 10, she developed a central line-associated BSI caused by CRAB identified by MALDI-TOF (Bruker Daltonics) directly from positive blood culture using a rapid protocol and then confirmed by culture plates.[8] The isolate was resistant to cefiderocol (11 mm zone diameter by disc diffusion, 30 μg cefiderocol disks, Liofilchem) and susceptible to colistin (MIC ≤0.5 mg/L, BD Phoenix™, Becton, Dickinson and/or Micronaut AST systems, Merlin Diagnostika GmbH) according to EUCAST pharmacokinetic/pharmacodynamic (PK/PD) breakpoints.[9] The patient started colistin 9 million IU IV loading dose, then 4.5 million IU q12h plus tigecycline 200 mg IV loading dose, then 100 mg q12h. Multiple attempts to discontinue colistin failed because of worsening clinical conditions with persistent isolation from skin lesions of CRAB and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA). Kidney function progressively declined and continuous renal replacement therapy (CRRT) was started. On Day 55, she also developed persistent candidaemia caused by *Candida parapsilosis* resistant to fluconazole, and caspofungin plus liposomal amphotericin B were started.

On Day 60, the patient had refractory hypoxia and worsening respiratory function. A CT scan revealed extensive bilateral multiple consolidations. Bronchoalveolar lavage (BAL) cultures grew CRAB and CRPA. The CRAB isolate was resistant to cefiderocol and colistin (MIC > 4 mg/L).

Given the lack of therapeutic options, we requested sulbactam/durlobactam, an investigational drug, [Entasis Therapeutics Inc., Waltham, MA, USA (Entasis Therapeutics)] as compassionate use. The MIC for sulbactam alone was 8 mg/L. Susceptibility to sulbactam/durlobactam was determined by Etest (bioMérieux, Inc, USA), supplied by Entasis Therapeutics, containing a gradient of sulbactam concentrations with the concentration of durlobactam fixed at 4 mg/L across the entire strip. Concurrent quality control (QC) procedures were performed by testing *Escherichia coli* ATCC 25922. MIC values were interpreted according to preliminary breakpoints provided by Entasis Therapeutics (susceptible MIC breakpoint ≤4 mg/L).[10] The CRAB isolate was susceptible to sulbactam/durlobactam (MIC 1.5 mg/L, Figure 1).

The patient started sulbactam/durlobactam 1.5/1.5 g every q6h infused over 3 h (dosage for CRRT) and continued colistin because of the concomitant CRPA isolation. In the following days, her clinical status improved and ventilatory support progressively decreased. Sulbactam/durlobactam was discontinued after 12 days. During the 30 day follow-up period after sulbactam/durlobactam end of therapy (EOT), CRPA was repeatedly isolated from BAL in the absence of worsening respiratory function, indicating persistent colonization of the respiratory tract by this organism; the patient had also relapsing candidaemia. Conversely, CRAB was eradicated and no further positive cultures from any site (skin lesions, BAL, blood and rectal swab) were detected. The patient was alive at 30 days from the sulbactam/durlobactam EOT. Informed consent was obtained and all the data were de-identified.

Figure 1. Etest for susceptibility of sulbactam/durlobactam against an *A. baumannii* isolate resistant to cefiderocol.

Genomic analysis

Whole Genome Sequencing (WGS) analysis revealed that the CRAB isolate belonged to ST2, according to the MLST Pasteur database.[11] The isolate harboured class C and D β-lactamase genes, including $bla_{\text{ADC-30}}$, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$. Several genes involved in cefiderocol resistance were investigated. A missense mutation in PBP3, which is the target of both cefiderocol and sulbactam, was detected, resulting in amino acid change N235K. Additionally, the TonB-dependent siderophore receptor gene *piuA* showed a frameshift mutation causing a premature stop codon (K384fs) and the *fepA* gene was interrupted by a transposon insertion P635-IS*Aba125* (IS*30* family).

Discussion

We have described a case of a severe infection caused by CRAB resistant to cefiderocol and colistin treated with sulbactam/durlobactam. Until now, two expanded-access cases of *A. baumannii* infection treated with sulbactam/durlobactam have been published. In the first case the patient received sulbactam/durlobactam in combination with cefiderocol,[12] and in the second case the patient received sulbactam/durlobactam in combination with meropenem.[13]

Resistance of CRAB isolates to cefiderocol represents a challenging issue. In the SIDERO-CR-2014/2016 surveillance study,

5% of CRAB were resistant to cefiderocol according to EUCAST breakpoints.[14] A microbiological study from China reported higher resistance rates (up to 35%) among CRAB isolates when CLSI breakpoints were used.[15] An observational clinical study showed that 8.5% of patients with CRAB infections developed resistance during or after exposure to cefiderocol.[7]

Mechanisms underlying resistance to cefiderocol in CRAB isolates involved several complex mechanisms. Our isolate harboured bla_{OXA} genes; this is not surprising as a recent surveillance study showed that the most prevalent carbapenemase gene detected in CRAB isolates was $bla_{OXA\text{-}23\text{-}like}$, the same one we found in our isolate.[16] More interesting, our strain also harboured some mutations in genes involved in iron transport that may contribute to the reduced susceptibility to cefiderocol. We found disruptions in genes encoding TonB-dependent siderophore receptors including the *piuA* gene and the *fepA* gene, which is orthologous to *pirA*. Similar mutations have been described in a recent study reporting an outbreak of cefiderocol-resistant CRAB among critically ill burn patients; in this study the resistant isolates harboured disrupted *piuA* and *pirA* genes that were intact in all susceptible isolates.[17] The *piuA* gene is involved in siderophore transport into the cell and its disruption has been reported as a mechanism of cefiderocol resistance in CRAB.[18] Finally, we found the mutation K235N in PBP3, the cefiderocol and sulbactam target, likely unrelated to cefiderocol resistance, although the same PBP3 mutation has been reported in another cefiderocol-non-susceptible isolate without other determinants of resistance.[19] Further studies are needed to explore the evolution and molecular characteristics of cefiderocol-resistant CRAB isolates.

We did not find other resistance mechanisms, including PER- and NDM-producing β-lactamases.[20] The absence of this latter carbapenemase is important considering that, in our region, NDM-producing Enterobacterales are endemic.[21] In our patient, we obtained microbiological eradication with sulbactam/durlobactam. Despite the occurrence of other infections due to other resistant organisms during the follow-up period, CRAB was absent from all other sites. It should be considered that our patient received sulbactam/durlobactam as the unique investigational active drug against the *A. baumannii* infection. The clinical success and microbiological eradication obtained with sulbactam/durlobactam seem to suggest that recommended dosages of the drug may be adequate in patients with VAP, but PK/PD studies are needed to confirm this hypothesis.

Data from the Phase 3 ATTACK trial comparing sulbactam/durlobactam plus imipenem/cilastatin versus colistin plus imipenem/cilastatin in patients with BSI and VAP by CRAB showed non-inferiority in 28 day all-cause mortality and overall trends favouring sulbactam/durlobactam (higher clinical cure rates and microbiologically favourable response).[22]

In conclusion, research for the optimal therapeutic strategy against CRAB is not over and further studies are needed to identify the best antibiotic treatment. Despite a narrow spectrum of activity, the investigational agent sulbactam/durlobactam is promising. Additional clinical studies are needed to confirm these preliminary results and identify the best antibiotic treatment regimen.

Acknowledgements

We thank Entasis Therapeutics for supplying sulbactam/durlobactam through its expanded access programme and to Dr David Altarac for support and guidance during the successful treatment of this patient. We also thank Dr Samir Moussa for supporting us in the interpretation of WGS of this isolate and Dr Alita Miller for review of the manuscript.

Funding

This study was supported by internal funding.

Transparency declarations

G.T. received honoraria for educational meetings by Shionogi. M.F. received unconditional grants from MSD and grants/or speaker honoraria from Angelini, Shionogi, Pfizer, Menarini, Gilead and Nordic Pharma. Declared conflicts of interest are outside the submitted work and did not affect the scientific objectivity of this study. Other authors have none to declare.

Data availability

The *A. baumannii* genome has been released under BioProject accession number PRJNA926509.

References

1 Falcone M, Tiseo G, Carbonara S *et al.* Mortality attributable to bloodstream infections caused by different carbapenem-resistant Gram negative bacilli: results from a nationwide study in Italy (ALARICO Network). *Clin Infect Dis* 2023; https://doi.org/10.1093/cid/ciad100

2 Tiseo G, Brigante G, Giacobbe DR *et al.* Diagnosis and management of infections caused by multidrug-resistant bacteria: guideline endorsed by the Italian Society of Infection and Tropical Diseases (SIMIT), the Italian Society of Anti-Infective Therapy (SITA), the Italian Group for Antimicrobial Stewardship (GISA), the Italian Association of Clinical Microbiologists (AMCLI) and the Italian Society of Microbiology (SIM). *Int J Antimicrob Agents* 2022; **60**: 106611. https://doi.org/10.1016/j.ijantimicag.2022.106611

3 Paul M, Daikos GL, Durante-Mangoni E *et al.* Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant Gram-negative bacteria: an open-label, randomised controlled trial. *Lancet Infect Dis* 2018; **18**: 391–400. https://doi.org/10.1016/S1473-3099(18)30099-9

4 Falcone M, Tiseo G, Nicastro M *et al.* Cefiderocol as rescue therapy for *Acinetobacter baumannii* and other carbapenem-resistant Gram-negative infections in intensive care unit patients. *Clin Infect Dis* 2021; **72**: 2021–4. https://doi.org/10.1093/cid/ciaa1410

5 Bassetti M, Echols R, Matsunaga Y *et al.* 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* 2021; **21**: 226–40. https://doi.org/10.1016/S1473-3099(20)30796-9

6 Pascale R, Pasquini Z, Bartoletti M *et al.* Cefiderocol treatment for carbapenem-resistant *Acinetobacter baumannii* infection in the ICU during the COVID-19 pandemic: a multicentre cohort study. *JAC Antimicrob Resist* 2021; **3**: dlab174. https://doi.org/10.1093/jacamr/dlab174

7 Falcone M, Tiseo G, Leonildi A *et al.* Cefiderocol- compared to colistin-based regimens for the treatment of severe infections caused by carbapenem-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2022; **66**: e0214221. https://doi.org/10.1128/aac.02142-21

8 Giordano C, Piccoli E, Brucculeri V *et al*. A prospective evaluation of two rapid phenotypical antimicrobial susceptibility technologies for the diagnostic stewardship of sepsis. *Biomed Res Int* 2018; **2018**: 6976923. https://doi.org/10.1155/2018/6976923

9 EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 11.0. 2021. https://www.eucast.org/clinical_breakpoints.

10 Karlowsky JA, Hackel MA, McLeod SM *et al*. *In vitro* activity of sulbactam-durlobactam against global isolates of *Acinetobacter baumannii-calcoaceticus* complex collected from 2016 to 2021. *Antimicrob Agents Chemother* 2022; **66**: e0078122. https://doi.org/10.1128/aac.00781-22

11 Diancourt L, Passet V, Nemec A *et al*. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS One* 2010; **5**: e10034. https://doi.org/10.1371/journal.pone.0010034

12 Zaidan N, Hornak JP, Reynoso D. Extensively drug-resistant *Acinetobacter baumannii* nosocomial pneumonia successfully treated with a novel antibiotic combination. *Antimicrob Agents Chemother* 2021; **65**: e0092421. https://doi.org/10.1128/AAC.00924-21

13 Holger DJ, Kunz Coyne AJ, Zhao JJ *et al*. Novel combination therapy for extensively drug-resistant *Acinetobacter baumannii* necrotizing pneumonia complicated by empyema: a case report. *Open Forum Infect Dis* 2022; **9**: ofac092. https://doi.org/10.1093/ofid/ofac092

14 Longshaw C, Manissero D, Tsuji M *et al*. *In vitro* activity of the siderophore cephalosporin, cefiderocol, against molecularly characterized, carbapenem-non-susceptible Gram-negative bacteria from Europe. *JAC Antimicrob Resist* 2020; **2**: dlaa060. https://doi.org/10.1093/jacamr/dlaa060

15 Wang Y, Li Y, Zhao J *et al*. Susceptibility of cefiderocol and other antibiotics against carbapenem-resistant, Gram-negative bacteria. *Ann Transl Med* 2022; **10**: 261. https://doi.org/10.21037/atm-22-889

16 Kohira N, Hackel MA, Ishioka Y *et al*. Reduced susceptibility mechanism to cefiderocol, a siderophore cephalosporin, among clinical isolates from a global surveillance programme (SIDERO-WT-2014). *J Glob Antimicrob Resist* 2020; **22**: 738–41. https://doi.org/10.1016/j.jgar.2020.07.009

17 Smoke SM, Brophy A, Reveron S *et al*. Evolution and transmission of cefiderocol-resistant *Acinetobacter baumannii* during an outbreak in the burn intensive care unit. *Clin Infect Dis* 2023; **76**: e1261–5. https://doi.org/10.1093/cid/ciac647

18 Abdul-Mutakabbir JC, Griffith NC, Shields RK *et al*. Contemporary perspective on the treatment of *Acinetobacter baumannii* infections: insights from the Society of Infectious Diseases Pharmacists. *Infect Dis Ther* 2021; **10**: 2177–202. https://doi.org/10.1007/s40121-021-00541-4

19 Yamano Y, Ishibashi N, Kuroiwa M *et al*. Characterisation of cefiderocol-non-susceptible *Acinetobacter baumannii* isolates from Taiwan. *J Glob Antimicrob Resist* 2022; **28**: 120–4. https://doi.org/10.1016/j.jgar.2021.12.017

20 Poirel L, Sadek M, Nordmann P. Contribution of PER-type and NDM-type β-lactamases to cefiderocol resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2021; **65**: e0087721. https://doi.org/10.1128/AAC.00877-21

21 Falcone M, Tiseo G, Antonelli A *et al*. Clinical features and outcomes of bloodstream infections caused by New Delhi metallo-β-lactamase-producing Enterobacterales during a regional outbreak. *Open Forum Infect Dis* 2020; **7**: ofaa011. https://doi.org/10.1093/ofid/ofaa011

22 Kaye KS, Shorr AF, Wunderink RG *et al*. Efficacy and safety of sulbactam-durlobactam versus colistin for the treatment of patients with serious infections caused by *Acinetobacter baumannii-calcoaceticus* complex: a multicentre, randomised, activecontrolled, phase 3, non-inferiority clinical trial (ATTACK). *Lancet Infect Dis* 2023: S1473-3099(23)00184-6. https://doi.org/10.1016/S1473-3099(23)00184-6 [Epub ahead of print].