## Transparency declarations

None to declare.

## Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

## References


**1** Tacconelli E, Carrara E, Savoldi A *et al*. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; **18**: 318–27. https://doi.org/10.1016/S1473-3099(17)30753-3

**2** Wada Y, Irekeola AA, E A R ENS, *et al*. Prevalence of vancomycin-resistant *Enterococcus* (VRE) in companion animals: the first meta-analysis and systematic review. *Antibiotics (Basel)* 2021; **10**: 138. https://doi.org/10.3390/antibiotics10020138

**3** Sacramento AG, Andrade AC D, Teotonio BN *et al*. WHO critical priority van-type vancomycin-resistant *Enterococcus* in dogs and cats. *Prev Vet Med* 2022; **202**: 105614. https://doi.org/10.1016/j.prevetmed.2022.105614

**4** CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-Third Edition: M100*. 2023.

**5** EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 13.1, 2023. https://www.eucast.org/clinical_breakpoints.

**6** Egan SA, Kavanagh NL, Shore AC *et al*. Genomic analysis of 600 vancomycin-resistant *Enterococcus faecium* reveals a high prevalence of ST80 and spread of similar *vanA* regions via IS*1216E* and plasmid transfer in diverse genetic lineages in Ireland. *J Antimicrob Chemother* 2022; **77**: 320–30. https://doi.org/10.1093/jac/dkab393

**7** Islam M, Sharon B, Abaragu A *et al*. Vancomycin resistance in *Enterococcus faecium* from the Dallas, Texas, area is conferred predominantly on pRUM-like plasmids. *mSphere* 2023; **8**: e0002423. https://doi.org/10.1128/msphere.00024-23

**8** Feßler AT, Scholtzek AD, Schug AR *et al*. Antimicrobial and biocide resistance among canine and feline *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates from diagnostic submissions. *Antibiotics (Basel)* 2022; **11**: 152. https://doi.org/10.3390/antibiotics11020152

**9** van Hal SJ, Ip CLC, Ansari MA *et al*. Evolutionary dynamics of *Enterococcus faecium* reveals complex genomic relationships between isolates with independent emergence of vancomycin resistance. *Microb Genom* 2016; **2**: e000048. https://doi.org/10.1099/mgen.0.000048

**10** Simjee S, White DG, McDermott PF *et al*. Characterization of Tn*1546* in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. *J Clin Microbiol* 2002; **40**: 4659–65. https://doi.org/10.1128/JCM.40.12.4659-4665.2002

**11** Sellera FP, Lincopan N. Zooanthroponotic transmission of high-risk multidrug-resistant pathogens: a neglected public health issue. *J Infect Public Health* 2019; **12**: 294–5. https://doi.org/10.1016/j.jiph.2018.12.013



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# *In vivo* development of cefiderocol resistance in carbapenem-resistant *Acinetobacter baumannii* associated with the downregulation of a TonB-dependent siderophore receptor, PiuA


**Jacqueline Findlay[1]*, Gabriele Bianco [2,3], Matteo Boattini [2,3] and Patrice Nordmann[1,4]**

*[1]Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland; [2]Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy; [3]Department of Public Health and Paediatrics, University of Torino, Turin, Italy; [4]Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland*

*Corresponding author. E-mail: Jacqueline.findlay@unifr.ch


Carbapenem-resistant *Acinetobacter baumannii* (CRAB) are a leading cause of nosocomial infections and subsequently present an urgent global public health threat.[1] OXA-23 is the most frequently reported carbapenemase in CRAB and such isolates are most often multidrug-resistant, leaving few therapeutic options.[1] Cefiderocol (FDC), a recently approved siderophore cephalosporin, represents a promising treatment option for CRAB infections, particularly since reported resistance rates in non-metallo-beta-lactamase-producing CRAB remain low.[2] FDC resistance in *A. baumannii* has previously been reported to be associated with mutations and/or deletions in the TonB-dependent receptors PiuA and PirA,[3] the carriage of certain beta-lactamases (e.g. MBLs and PER-type),[4] and mutations within the penicillin binding protein PBP-3, the main target of FDC.[3] In this study, we describe the *in vivo* development of FDC resistance following treatment, mediated by a previously undescribed mutation in the promoter region of TonB-dependent receptor, PiuA.

An 80-year-old woman was admitted to the ICU of the University Hospital 'Città della salute e della scienza di Torino' (Turin, Italy) due to a severe burn injury (20% TBSA). Empirical antibiotic therapy with piperacillin/tazobactam plus amikacin was started. On day 52, she presented with fever and elevation of inflammatory markers. Blood cultures were positive for an FDC-susceptible CRAB isolate (AB1). FDC plus fosfomycin were started and the patient clinically improved. After 10 days, she presented with fever and an FDC-resistant CRAB isolate was isolated from a blood culture (AB2). FDC treatment was stopped,

**Table 1.** Genotypic and phenotypic characteristics of isolates AB1 and AB2

| Isolate | ST | Beta-lactamase genes | Other resistance genes | MICs (mg/L) FDC | IPM | MEM | SNPs | Relative *piuA* expression |
|---|---|---|---|---|---|---|---|---|
| AB1 | 2 | $bla_{OXA-23}$, $bla_{OXA-66}$, $bla_{ADC-293}$ | *armA*, *aph(3')-Ib*, *aph(6)-Id*, *msr(E)*, *mph(E)*, *sul2*, *tetB* | 1 | 128 | 64 | NA | 1 |
| AB2 | 2 | $bla_{OXA-23}$, $bla_{OXA-66}$, $bla_{ADC-293}$ | *armA*, *aph(3')-Ib*, *aph(6)-Id*, *msr(E)*, *mph(E)*, *sul2*, *tetB* | 32 | 128 | 64 | 3 nt deletion upstream of *piuA* | 0.35 |

FDC, cefiderocol; IPM, imipenem; MEM, meropenem.

fosfomycin plus ampicillin/sulbactam were started, and the patient clinically improved. Susceptibility testing, performed by broth microdilution and interpreted according to EUCAST guidelines,[5] showed that isolates AB1 and AB2 exhibited FDC MICs of 1 and 32 mg/L respectively, and were additionally resistant to both imipenem and meropenem (Table 1). Isolates were subject to Illumina WGS and subsequent analyses were performed using the Center for Genomic Epidemiology Platform (https://www.genomicepidemiology.org/) as previously described.[6] Both isolates belonged to ST2 (according to the Pasteur MLST scheme)[7] and harboured an identical resistance gene content, including a carbapenemase encoding gene $bla_{OXA-23}$ and the intrinsic beta-lactamase genes $bla_{OXA-66}$ and $bla_{ADC-293}$. The isolates additionally harboured a gene encoding the aminoglycoside-modifying enzyme ArmA. ST2 *A. baumannii* is the most dominant type globally (a member of Global Clone 2) and is considered a high-risk clone, often associated with the production of carbapenemases, particularly OXA-23.[1] SNP analyses using Snippy (https://github.com/tseemann/snippy) identified that the only difference between both isolates was a 3 nt deletion, located 35 bp upstream of a TonB-dependent siderophore receptor gene, *piuA*. This deletion was located between the −35 and −10 elements of a predicted σ70 promoter, resulting in a 'weaker' promoter sequence, predicted to result in decreased RNA polymerase binding. To investigate the impact of this deletion on gene expression quantitative RT–QPCR was performed on both isolates, AB1 and AB2. Briefly, isolates were grown in iron-depleted Mueller–Hinton media to mid-exponential growth phase ($OD_{600}$ ∼0.5), before RNA extraction using the Monarch® Total RNA Miniprep Kit (New England Biolabs), DNA digestion using TURBO™ DNase (ThermoFisher) and cDNA synthesis using LunaScript (New England Biolabs). Quantitative PCR was performed on a Qiagen RotorGene using the Promega SYBR green PCR mix and the following primers: for *piuA*, AB_piuA_qF (5′-CAGTTGGTGGCAG CATCAAT-3′) and AB_piuA_qR (5′-TGCTGCAATGCCATTTCCAA-3′), and for *rpoB*, AB_rpoB_qF (5′-ACGCCTAAAGGTGAAACTCAG TTAA-3′) and AB_rpoB_qR (5′-GTACCAGATGGAACACGTAAAG ATG-3′). All assays were performed on three biological replicates, and the delta delta Ct method was used to calculate relative expression levels. Analyses identified that the *piuA* was significantly downregulated (∼3-fold reduction; Table 1) in isolate AB2, and subsequently this was determined to be the mechanism responsible for elevated the FDC MICs. To investigate the fitness cost of this insertion, growth curves were performed on three biological replicates for each isolate, in iron-depleted Mueller–Hinton media over an 8-hour period. This showed the promoter mutation in strain AB2 resulted in a significant fitness cost, ranging from a 15% to 30% reduction in growth, over the early to late exponential growth phase ($OD_{600}$ 0.3–0.8), relative to AB1. This can be explained by the fact that iron is essential for cell function, and the limited uptake through a functional PiuA could be expected to compromise cell fitness.[8]

In conclusion, this study illustrated the *in vivo* development of FDC resistance in an OXA-23-producing ST2 *A. baumannii* isolate, as a direct consequence of FDC therapy. A single mutation event was sufficient to result in a 32-fold increase in FDC MIC although this resistance was shown to come at a fitness cost to the bacterium. This demonstrates the ability of *A. baumannii* to mutate and adapt to cope with challenge by FDC, and underlines that despite being a promising therapeutic option for the treatment of CRAB, FDC should be prescribed sparingly to preserve its use. Studies on the evaluation of the most effective combinations for treating infections due to multidrug-resistant *A. baumannii* infections are ongoing. They include drugs such as FDC, ampicillin/sulbactam sulbactam/durlobactam colistin and rifampicin. The use of FDC in combination with another antibiotic is debatable for preventing emerging resistance to FDC, and certainly requires further investigation.

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## Transparency declarations

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## References

**1** Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. *Microb Genom* 2019; **5**: e000306. https://doi.org/10.1099/mgen.0.000306

**2** Seifert H, Müller C, Stefanik D *et al*. In vitro activity of cefiderocol against a global collection of carbapenem-resistant *Acinetobacter baumannii* isolates. *Antibiotics (Basel)* 2023; **12**: 1172. https://doi.org/10.3390/antibiotics12071172

**3** Malik S, Kaminski M, Landman D *et al*. Cefiderocol resistance in *Acinetobacter baumannii*: roles of β-lactamases, siderophore receptors,

and penicillin binding protein 3. *Antimicrob Agents Chemother* 2020; **64**: e01221-20. https://doi.org/10.1128/AAC.01221-20

**4** 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

**5** European Committee on Antimicrobial Susceptibility Testing. Clinical Breakpoint Table v. 13.1. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.1_Breakpoint_Tables.pdf.

**6** Findlay J, Perreten V, Poirel L *et al*. Molecular analysis of OXA-48-producing *Escherichia coli* in Switzerland from 2019 to 2020. *Eur J Clin Microbiol Infect Dis* 2022; **41**: 1355–60. https://doi.org/10.1007/s10096-022-04493-6

**7** 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

**8** Kramer J, Özkaya Ö, Kümmerli R. Bacterial siderophores in community and host interactions. *Nat Rev Microbiol* 2020; **18**: 152–63. https://doi.org/10.1038/s41579-019-0284-4

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# Modification of the penicillin-binding-protein 3 as a source of resistance to broad-spectrum cephalosporins in *Escherichia coli*

**Samanta Freire[1], Jacqueline Findlay[1,2], Eva Gruner[3], Vera Bruderer[3], Patrice Nordmann[1,2] and Laurent Poirel [1,2]***

[1]*Faculty of Science and Medicine, Medical and Molecular Microbiology, University of Fribourg, Fribourg, Switzerland;* [2]*Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland;* [3]*Medica—Medizinische Laboratorien Dr F. Kaeppeli AG, Zürich, Switzerland*

*Corresponding author. E-mail: laurent.poirel@unifr.ch

Multidrug-resistant Gram-negative bacteria are one of the major threats to human health, largely due to the dissemination of some high-risk successful multidrug-resistant clones.[1] Of particular concern is the resistance to ß-lactams, because those antibiotics are the most extensively used antimicrobial ones, accounting for half of all prescriptions in Europe.[2] Resistance to broad-spectrum cephalosporins is of particular concern, as related infections require usage of carbapenems considered as last-resort antibiotics supposed to be preserved. Although the production of β-lactamases is the most common mechanism of resistance to broad-spectrum β-lactams in Gram-negative bacteria, and particularly in Enterobacterales, other mechanisms have also been reported including efflux, permeability changes and modification of PBP targets that contribute to this resistance.[3]

Our study was initiated by the isolation in March 2023 of an *Escherichia coli* strain recovered from a urine sample of an 86-year-old female patient hospitalized in Zürich, Switzerland, suffering from recurrent urinary tract infections. Corresponding antibiotic regimen were either cotrimoxazole or fosfomycin, but no ß-lactam has been used. Susceptibility testing by disc diffusion and broth microdilution showed that this isolate was resistant to broad-spectrum cephalosporins (ceftazidime, cefotaxime, cefepime) and to aztreonam, but remained susceptible to ceftazidime-avibactam and carbapenems, according to the EUCAST 2023 breakpoints.[4] In addition, it was resistant to fluoroquinolones, nitrofurantoin, cotrimoxazole and fosfomycin.

Multilocus sequence typing performed as described in ref.[5] identified that strain as belonging to ST1193, that has been reported as an emerging high-risk multidrug-resistant global clone.[1] Resistance to broad-spectrum cephalosporins (ceftazidime, cefotaxime, cefepime) could not be explained by common mechanisms of resistance such as broad-spectrum ß-lactamases. Indeed, the Rapid ESBL NP test remained negative and PCR assays for all ESBL-encoding genes also remained negative.[6] Likewise, PCR for all known AmpC β-lactamase encoding genes remained negative.

Whole-genome sequencing was therefore performed, using a short-read technology (MiSeq platform, Illumina, San Diego, CA, USA),[7] and allowed the identification of mutations in *gyrA*/*parC* (*gyrA* S83L, D87N; *parC* S80I), responsible for fluroquinolone resistance,[1] and an 8-bp insertion and subsequent disruption of *nfsA*, conferring resistance to nitrofurantoin.[8] Regarding β-lactam resistance, the gene encoding the narrow-spectrum TEM-1 was the sole ß-lactamase gene identified, that explained the resistance to penicillins but not to the broad-spectrum cephalosporins.[9] However, a four amino-acid insertion (YRVP) just after the amino-acid residue 333 (close to the β-lactam binding pocket) of the PBP-3 sequence was identified.

To investigate the role of this PBP-3 insertion in the resistance to cephalosporins, a wild-type PBP-3 was amplified from *E. coli* ATCC25922 reference strain using primers PBP3_F (5′-CCACGGA AAAGCTGCAAATG-3′) and PBP3_R (5′-CATCGGTCGCCTCATCTT TC-3′) and cloned into plasmid pCR-Blunt II-TOPO,[7] then the corresponding recombinant plasmid transformed into the *E. coli* clinical