Rapid development of cefiderocol resistance in carbapenem-resistant *Enterobacter cloacae* during therapy is associated with heterogeneous mutations in the catecholate siderophore receptor cirA.

Sabrina Klein¹*, Sébastien Boutin¹*, Kaan Kocer³, Mascha O. Fiedler², Dominic Störzinger³, Markus A. Weigand², Benjamin Tan², Daniel Richter², Christian Rupp⁴, Markus Mieth⁵, Arianeb Mehrabi⁵, Thilo Hackert⁵, Stefan Zimmermann¹, Klaus Heeg¹, Dennis Nurjadi¹.

* equal contribution

¹ Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University Hospital, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany

² Department of Anesthesiology, Heidelberg University Hospital, Im Neuenheimer Feld 420, 69120 Heidelberg, Germany

³ Pharmacy Department, Heidelberg University Hospital, Im Neuenheimer Feld 670, 69120 Heidelberg, Germany

⁴ Department of Internal Medicine IV, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

⁵ Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Im Neunheimer Feld 420, 69120 Heidelberg, Germany

© The Author(s) 2021. Published by Oxford University Press for the Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
correspondence:

Dennis Nurjadi, MD, Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University Hospital, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany
e-mail: dennis.nurjadi@uni-heidelberg.de, phone +49 6221 5637817
Abstract

We report a case of resistance development towards cefiderocol in a patient with intra-abdominal and bloodstream infections caused by carbapenemase-producing Enterobacter cloacae within 21 days of cefiderocol therapy. Whole genome sequencing revealed heterogeneous mutations in the cirA gene, encoding a catecholate siderophore receptor, conferring phenotypic resistance to cefiderocol.

Keywords: cefiderocol, cirA siderophore receptor, Enterobacter cloacae, antibiotic resistance, carbapenemase
The emergence and spread of carbapenem resistance in Gram-negative bacteria is an on-going global threat, limiting therapeutic options for severe infections [1], and are one of the leading causes of morbidity and mortality in vulnerable patients [2]. Cefiderocol is a novel synthetic conjugate siderophore cephalosporin, recently approved by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Cefiderocol exploits the iron transporters of the bacterial outer membrane by binding with iron molecules to form a siderophore complex, entering the periplasmic compartment and unfold its antibacterial activity by inhibiting the cell wall synthesis. Cefiderocol exhibited stability against hydrolysis by all carbapenemases, including metallo-β-lactamases (MBLs) and therefore is approved for the treatment of infections with aerobic Gram-negative bacteria with limited therapy options [3].

The redundancy of the bacterial TonB-dependent iron transport system was expected to be a hurdle for rapid resistance development [4]. Nonetheless, the acquisition of cefiderocol resistance has been reported. Yet, the underlying mechanisms remain unclear [5].

Here, we report the in vivo development of cefiderocol resistance within three weeks after therapy initiation in a critically ill patient with bloodstream and intra-abdominal infection caused by carbapenem-resistant (CR) Enterobacter cloacae. Comparative genomics analysis using whole-genome sequencing (WGS) was performed to identify potential mechanisms associated with the phenotypic resistance to cefiderocol.

**Clinical Case**

A 58-year-old male patient presented with liver cirrhosis 10 years after initial liver transplantation. Following successful othotopic liver re-transplantation, he developed a complicated clinical course with leakage of the bile duct, insertion of a percutaneous transhepatic drainage, infection with vancomycin-resistant Enterococcus faecium (VREfm) and formation of biloma. On day 68 following transplantation, blood cultures were positive for Klebsiella pneumoniae and CR-Enterobacter cloacae. Two carbapenemase genes encoding for New Delhi metallo-β-lactamase (NDM) and
oxacillinase, OXA-48 were detected in the CR- \textit{E. cloacae} by PCR. Antimicrobial therapy with meropenem and colistin was initiated. CR- \textit{E. cloacae} and VREfm were continuously cultured from the bile drainage. Due to severe neurological side-effects, the acquisition of colistin resistance and the lack of alternatives, antimicrobial therapy was switched from meropenem and colistin to cefiderocol on day 75 (Figures 1a and b). The follow-up blood cultures on day 78, 83 and 93 (day 4, 9 and 19 after the first cefiderocol administration) remained negative, suggesting therapeutic success. However, cultures from the bile drainage remained positive for CR- \textit{E. cloacae} and VREfm, and treatment of VREfm was switched back to linezolid. CT scan demonstrated again multiple biloma and hepatic abscesses. Caspofungin was initiated because of the detection of \textit{Candida glabrata} and \textit{Candida albicans} in the bile drainage. Due to persistent VREfm infection, daptomycin was again added to the regimen. On day 21 of cefiderocol therapy, cefiderocol-resistant CR- \textit{E. cloacae} was detected in the blood culture. Despite clinical worsening, no potentially curative surgical option was technically possible due to the lacking arterial vascularization and consecutive bile duct necrosis as well as intrahepatic abscesses; leading to exitus letalis on day 107. Detailed information on the clinical course is given in the Supplementary material.

**Methods**

Diagnostic routine microbiology was performed as described previously [6]. Antibiotic susceptibility testing (AST) was interpreted according to EUCAST clinical breakpoints v10.0 . AST for cefiderocol was determined by microdilution (Sensititre, Thermo Fisher Scientific) according to the manufacturer’s protocol. Since the upper limit for the commercially available Sensititre AST is 8mg/L for cefiderocol, a broth microdilution (range 0.5mg/L to >256mg/L) using iron-depleted cation-adjusted Mueller-Hinton broth was performed according to the published protocol by Hackel and colleagues [7]. For whole genome sequencing, DNA of \textit{E. cloacae} isolates from blood culture was isolated and sequenced on the MiSeq Illumina platform (short-read sequencing 2x300bp), as
described previously [6]. Details on data analysis and data availability (PRJNA705064) are provided in the Supplementary Material.

Results

The phenotypic resistance profile is displayed in Figure 1b. All *E. cloacae* isolates belonged to ST96 and harbored *bla*<sub>NDM-5</sub> and *bla*<sub>OXA-48</sub>. Minimum inhibitory concentrations (MIC) of cefiderocol for blood culture isolates etcl_1 was 4mg/L, and 2mg/L for etcl_2, 3 and 4. These isolates are considered susceptible based on the CLSI breakpoints, but etcl_1 is considered resistant according to the EUCAST clinical breakpoints. AST of subsequent isolates, etcl_5 to 9, revealed a considerably higher MIC of ≥ 256 mg/L (Figure 1c). Etcl_3 was phenotypically colistin resistant, but neither mutation nor acquisition of genes associated with colistin resistance was detected.

Blood culture isolates were cryo-preserved and we performed WGS on all nine isolates detected in the blood cultures of this patient before and during cefiderocol therapy. Alignment of the core genome (4811 genes, 4575108 nucleotides) revealed close genetic relationship with single nucleotide polymorphism (SNP) ranging between 0 to 9 among the patient’s isolates, indicating a common clonal origin (Figure 1c).

Resistance to cefiderocol has been reported to be associated with alterations of the intrinsic AmpC and siderophore receptors [8]. In the presented case, the chromosomal AmpC of all isolates were identical, so that AmpC-mediated resistance was unlikely. To find a potential resistance mechanism, we analyzed the SNPs and deletion distribution among the genes of the core-genome and compared them between cefiderocol-susceptible and cefiderocol-resistant isolates. Comparison of the draft genomes between the cefiderocol-susceptible (etcl_1 to 4) and resistant (etcl_5 to 9) isolates revealed alterations in only one gene, *cir*A, which encodes a TonB-dependent catecholate siderophore receptor (Supplementary Figure S1).
In isolates etcl_5, _6, _7 and _9, we detected various mutations in the CirA gene between 1089 and 1595 bp, which were absent in all isolates prior to cefiderocol exposure (Figure 1c). *In silico* protein translation revealed truncation of the CirA protein in the isolates with the high-level cefiderocol resistance (Figure 1c, Supplementary material). In isolate etcl_8, we detected an insertion of an IS5-like Transposon, which also resulted in truncation of the CirA protein (Figure 1c and Supplementary Figure S2 and S3).

**Discussion**

Cefiderocol is a promising agent for the treatment of Gram-negative bacteria with limited therapeutic options [3, 9]. Although the development of cefiderocol resistance has been described, reduced susceptibility towards cefiderocol in Enterobacterales is not a common encounter [10]. To the best of our knowledge, this case is the first report on the development of high-level cefiderocol resistance during cefiderocol therapy. In the present case, cefiderocol resistance in *E. cloacae* developed rapidly within 21 days of therapy. WGS identified heterogeneous functional alterations in the catecholate siderophore receptor gene, cirA. Indeed, the C-3 side chain of cefiderocol consists of a catechol group, which binds to iron molecules to enter the periplasmic compartment through a catecholate iron transporter, demonstrating the importance of the catecholate siderophore receptor as the entry mechanism for cefiderocol in *E. cloacae*. Furthermore, in the annotated draft genome of *E. cloacae* isolates in the present case, we identified CirA as the only catecholate receptor present, explaining the impact of the mutation of this gene.

Furthermore, the mutation observed in this case were heterogeneous but located in a narrow window from 1089 to 1595 bp of the genes, indicating a potential mutational hot-spot leading to a highly truncated protein and non-functional receptor. The potential significance of CirA in cefiderocol’s mode of entry has been previously investigated for *Escherichia coli* by Ito and
colleagues [4]. In contrast to our case, a deletion of the cirA gene had little impact on the cefiderocol MIC, whereas an additional deletion of the ferrichrome receptor gene fluA led to a significant increase of the MIC towards cefiderocol. Based on this observation and other published studies [4, 11], a redundancy of the TonB-dependent ferric transport system in the outer membrane of Gram-negative bacteria such as Pseudomonas aeruginosa or E. coli may prevent rapid development of resistance to cefiderocol. The rapid development of resistance in the presented case might have been favored by elevated initial cefiderocol MICs, caused by the production of metallo-β-lactamase. Generally, the cefiderocol MIC in isolates producing metallo-β-lactamases remain below the threshold for resistance [12, 13], therefore cefiderocol can still be considered as a viable option to treat infections with carbapenemase-producing Gram-negatives. In E. cloacae complex, the development of cefiderocol resistance following cefepime exposure has been reported as a result of an amino acid deletion in the R2-loop of AmpC beta-lactamase [14]. The alteration of the chromosomal AmpC beta-lactamase is a general response directed to the beta-lactam antibiotic class following beta-lactam exposure and hence it is not a mutation directly interfering with cefiderocol or its delivery mode so that cefiderocol resistance in this case can be considered as a collateral effect.

Our findings indicated that high-level resistance could be acquired rapidly through mutations of the CirA siderophore receptor during cefiderocol therapy. However, it remains unclear whether the rapid development of resistance is a feature of Enterobacter spp. or if the presence of one or both carbapenemases increases the propensity for resistance acquisition. As a limitation, we did not perform molecular validation to confirm the direct effect of cirA deletion on cefiderocol susceptibility. Further investigations are needed to study the role and diversity of the siderophore receptor repertoire in Gram-negative bacteria. The acquisition of cefiderocol resistance in E. cloacae during therapy is alarming and should be closely monitored.
NOTES

Acknowledgement

We would like to thank Selina Hassel, Nicole Henny, Suzan Leccese and Delal Sahin for their excellent technical assistance. Bacterial isolates were collected and sequenced as part of the MDRO surveillance program at our university hospital. Individual informed consent was waived after consulting the local ethics committee for the scientific use of anonymized patient and clinical data, along with bacterial genomic data (S474/2018).

Funding

none

Potential conflict of interest

S.Z. reports non-financial support from Shionogi Germany, is a member of the cefiderocol advisory board of Shionogi Germany, and received honorarium for Shionogi board meeting in October 2020.

M.A.W. provided presentations for and is an advisory board member of and received speaker honorarium and non-financial support from Shionogi, Pfizer, MSD, Eumedica, Gilead. All other authors have no conflict of interest to declare.
References

1. Kelly AM, Mathema B, Larson EL. Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. Int J Antimicrob Agents 2017; 50(2): 127-34.

2. Martin A, Fahrbach K, Zhao Q, Lodise T. Association Between Carbapenem Resistance and Mortality Among Adult, Hospitalized Patients With Serious Infections Due to Enterobacteriaceae: Results of a Systematic Literature Review and Meta-analysis. Open Forum Infect Dis 2018; 5(7): ofy150.

3. Sato T, Yamawaki K. Cefiderocol: Discovery, Chemistry, and In Vivo Profiles of a Novel Siderophore Cephalosporin. Clin Infect Dis 2019; 69(Suppl 7): S538-S43.

4. Ito A, Sato T, Ota M, et al. In Vitro Antibacterial Properties of Cefiderocol, a Novel Siderophore Cephalosporin, against Gram-Negative Bacteria. Antimicrob Agents Chemother 2018; 62(1).

5. Naseer S, Weinstein EA, Rubin DB, et al. US Food and Drug Administration (FDA): Benefit-Risk Considerations for Cefiderocol (Fetroja(R)). Clin Infect Dis 2020.

6. Kocer K, Boutin S, Dalpke AH, Heeg K, Mutters NT, Nurjadi D. Comparative genomic analysis reveals a high prevalence of inter-species in vivo transfer of carbapenem-resistance plasmids in patients with haematological malignancies. Clin Microbiol Infect 2020; 26(6): 780 e1-e8.

7. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. Diagn Microbiol Infect Dis 2019; 94(4): 321-5.

8. Shields RK, Iovleva A, Kline EG, Kawai A, McElheny CL, Doi Y. Clinical evolution of AmpC-mediated ceftazidime-avibactam and cefiderocol resistance in Enterobacter cloacae complex following exposure to cefepime. Clin Infect Dis 2020.

9. Bonomo RA. Cefiderocol: A Novel Siderophore Cephalosporin Defeating Carbapenem-resistant Pathogens. Clin Infect Dis 2019; 69(Suppl 7): S519-S20.

10. Mushtaq S, Sadouki Z, Vickers A, Livermore DM, Woodford N. In Vitro Activity of Cefiderocol, a Siderophore Cephalosporin, against Multidrug-Resistant Gram-Negative Bacteria. Antimicrob Agents Chemother 2020; 64(12).
11. Nikaido H, Rosenberg EY. Cir and Fiu proteins in the outer membrane of Escherichia coli catalyze transport of monomeric catechols: study with beta-lactam antibiotics containing catechol and analogous groups. J Bacteriol 1990; 172(3): 1361-7.

12. Kazmierczak KM, Tsuji M, Wise MG, et al. In vitro activity of cefiderocol, a siderophore cephalosporin, against a recent collection of clinically relevant carbapenem-non-susceptible Gram-negative bacilli, including serine carbapenemase- and metallo-beta-lactamase-producing isolates (SIDERO-WT-2014 Study). Int J Antimicrob Agents 2019; 53(2): 177-84.

13. Delgado-Valverde M, Conejo MDC, Serrano L, Fernandez-Cuenca F, Pascual A. Activity of cefiderocol against high-risk clones of multidrug-resistant Enterobacterales, Acinetobacter baumannii, Pseudomonas aeruginosa and Stenotrophomonas maltophilia. J Antimicrob Chemother 2020; 75(7): 1840-9.

14. Kawai A, McElheny CL, Iovleva A, et al. Structural Basis of Reduced Susceptibility to Ceftazidime-Avibactam and Cefiderocol in Enterobacter cloacae Due to AmpC R2 Loop Deletion. Antimicrob Agents Chemother 2020; 64(7).
**Figure 1.** (a) Timeline of events and antimicrobial therapy from the first detection of carbapenem-resistant *Enterobacter cloacae* until *exitus letalis*. High-level cefiderocol-resistant was first detected on day 21 after therapy initiation (day 68 after liver re-transplantation). Dark grey bars indicate antimicrobial therapy duration. Dosages: Daptomycin i.v. 700mg every 24 hours, meropenem i.v. 2g initial bolus followed by 2g every 8 hours with prolonged infusion time, colistin i.v. 9 Mio. loading dose followed by 2,5 Mio. every 12 hours for 3 days and 4,5 Mio. every 12 hours for another 3 days, cefiderocol i.v. 2g initial bolus followed by 1g every 8 hours (dose adjusted to renal clearance) for 26 days and 2g every 8 hours for 6 days, linezolid i.v. 600mg every 12 hours, caspofungin i.v. 70mg on the first day and then 50mg every 24 hours. (b) Phenotypic resistance profile of all *Enterobacter cloacae* blood culture isolates. (c) Phylogeny by single nucleotide polymorphisms (SNP) of *E. cloacae* isolates indicated close clonal relationship (ST96, 0 to 9 SNP). The isolates etcl_6 to 9 exhibited high-level resistance to cefiderocol (>256 mg/L).

Abbreviations; FDC-S=cefiderocol-susceptible, FDC-R=cefiderocol-resistant, MLST=multi-locus sequence typing, MIC=minimum inhibitory concentration in mg/L, S=susceptible, I=intermediate, R=resistant.
A negative blood culture was observed in E. cloacae FDC-S and FDC-R strains. The treatment with antibiotics such as cefiderocol, colistin, daptomycin, linezolid, meropenem, and caspofungin was administered from days 68 to 103 post transplantation.

**Diagram a** shows the timeline of antibiotic administration and the presence of the bacteria.

**Diagram b** illustrates the MIC values for various antibiotics, with cefiderocol showing the greatest activity.

**Diagram c** provides the MIC values and the specific mutations in the CirA protein. The MIC values are as follows:

- cefiderocol: 2
- imipenem: 4
- piperacillin/tazobactam: >256
- cefotaxime: 256
- ceftazidime: 2
- cephalosporins: >256
- colistin: 256
- gentamicin: 2
- tobramycin: 256
- amikacin: 2
- aztreonam: 2
- fosfomycin: 2
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256
- imipenem/relebactam: 256
- ciprofloxacin: 256
- trimethoprim/sulfamethoxazole: >256
- gentamicin: 256
- tobramycin: 256
- amikacin: 256
- aztreonam: 256
- fosfomycin: 256
- cefepime: >256
- ceftriaxone: 2
- ceftazidime/avibactam: 256
- meropenem/vaborbactam: 256