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# *In vitro* and *in vivo* activity of cefiderocol against *Burkholderia cepacia* complex clinical isolates

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## ABSTRACT

**Background:**
*Burkholderia* species have been recognized as difficult to treat pathogens causing a range of infections in immunocompromised patients. *Burkholderia* possess a number of intrinsic mechanisms leading to antibiotic resistance and antimicrobial susceptibility is variable which can impact treatment options. In this study, we examined the *in vitro* activity and *in vivo* efficacy of cefiderocol (CFDC), a novel parenteral siderophore cephalosporin with potent activity against a diverse collection of *B. cepacia* complex isolates including carbapenem-resistant strains.

**Materials and methods:**
A total of 164 isolates of *B. cepacia* complex (94 *B. cepacia*, 34 *B multivorans*, 33 *B. cenocepacia*, 2 *B. vietnamensis*, and 1 *B. dolosa*) collected in North America and Europe (SIDERO-WT 2014 to 2016) were examined. MICs were determined for CFDC, cefepime (FEP), ceftazidime-avibactam (CZA), ceftolozane-tazobactam (C/T), ciprofloxacin (CIP), colistin (CST), and meropenem (MEM) by broth microdilution method according to CLSI guidelines. Cefiderocol was tested in iron-depleted cation-adjusted Mueller Hinton broth (ID-CAMHB). *In vivo* efficacy of CFDC, FEP, CZA, MEM, and CST was evaluated using neutropenic murine systemic infection model. CFDC resistant strains were examined by whole genome sequence to identify potential mechanisms of resistance.

**Results:**
MIC$_{90}$s of CFDC, MEM, CZA, and C/T against 164 isolates were 0.25, 16, 8, and 32 mg/L, respectively. MIC$_{90}$ of cefiderocol against *B. cenocepacia*, *B. cepacia*, *B. dolosa*, *B. multivorans*, and *B. vietnamensis* were 0.03, 0.12, 1, 32, and 0.015 mg/L, respectively. CFDC was the most active compound among the tested compounds, although 6/34 isolates of *B. multivorans* and 1/94 *B. cepacia* showed non-susceptibility to CFDC. The ED$_{50}$ of CFDC against *B. cepacia* SR25744 (MIC: 0.016 mg/L) was 2.11 mg/kg/dose. The ED$_{50}$s against FEP and CZA were >100 mg/kg. Genotypic analysis of CFDC non-susceptible *B. multivorans* illustrated that 3 of 5 isolates exhibited disruptions in homologs of the catecholate siderophore receptor (*fiu*-like) genes.

**Conclusions:**
CFDC demonstrated potent *in vitro* activity against *B. cepacia* complex, with greater than 95.7 % of tested isolates having MIC values ≤4 mg/L. These findings indicate that CFDC has high potential for treating infections caused by these difficult to treat organisms.

## INTRODUCTION

Cefiderocol is a novel parenteral catechol-substituted siderophore cephalosporin that is active against carbapenem-resistant Gram-negative bacteria (1, 2). In this study, we evaluated the *in vitro* activity of CFDC and comparators against *Burkholderia cepacia* complex collected from a multi-national surveillance studies of Gram negative clinical isolates. We also evaluated *in vivo* activity of CFDC against *B. cepacia* in a murine systemic infection model.

## MATERIALS AND METHODS

### Test organisms
All *B. cepacia* complex isolates (N = 164) were used, which were obtained from surveillance studies (SIDERO-WT-2014, SIDERO-WT-2015, and SIDERO-WT-2016). In SIDERO-WT-2014 study, 12 strains of *B. cepacia* complex were isolated among a total of 9205 Gram-negative pathogens isolated in 2014 Nov to 2015 Oct. In SIDERO-WT-2015 study, 88 strains of *B. cepacia* complex were isolated among a total of 8954 Gram-negative pathogens isolated in 2015 Nov to 2016 Oct. In SIDERO-WT-2016 study, 63 strains of *B. cepacia* complex were isolated among a total of 10470 Gram-negative pathogens isolated in 2016 Nov to 2017 Oct from North America and EU countries. *B. cepacia* SR25744, which was isolated in Japan, was used for the evaluation using *in vivo* systemic infection model. Tables 1 and 2 show the geographic region and body location information of test strains in this study.

### Compound
Cefiderocol (CFDC), cefepime (FEP), ceftazidime/avibactam (CZA), ceftolozane/tazobactam (C/T), ciprofloxacin (CIP), colistin (CST), and meropenem (MEM) were used.

### MIC determination
Broth microdilution testing was done according to Clinical and Laboratories Standard Institute (CLSI) guidelines (3, 4). To test cefiderocol, the CLSI approved methodology using iron-depleted cation-adjusted Mueller-Hinton Broth (ID-CAMHB) was used. The cefiderocol MIC was read as the first drug well in which growth was significantly reduced (i.e., a button of <1 mm or light/faint turbidity) relative to the growth observed in growth control. The recommended CLSI reference quality control (QC) *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used throughout the testing.

### Whole genome sequence
Whole genome sequencing was performed on 5 bacterial isolates of *B. multivorans* which showed non-susceptibility to CFDC with MIC of >4 mg/L. All genotypic analyses were performed using the CLC genomics workbench (Version 11, QIAgen).

### Neutropenic murine systemic infection model
ICR male mice were rendered neutropenic by intraperitoneal injection of cyclophosphamide (Shionogi, Osaka, Japan) with 150 and 100 mg/kg at 4 and 1 days before infection, respectively. Neutropenic mice were infected by intraperitoneal injection of 0.5 mL of bacterial suspension with 5% gastric mucin. The challenge dose was approximately 1.2 × 10$^5$ CFU/mouse. CFDC, FEP, CZA, MEM/Cilastatin were administered subcutaneously at 1, 3 and 5 hours (hour) after infection. CST was administered subcutaneously at 1 and 5 hours after infection. The 50% effective doses (ED$_{50}$s) of CFDC and comparators were calculated by the logit method using the survival numbers at each dose 7 days after infection. Windows SAS program (SAS® Version 9.4) was used for calculating ED$_{50}$s.

### Table 1. Body site distribution of tested isolates

| Body Location | | Number of test strain | Ratio (%) | Total (%) |
|---|---|---|---|---|
| RTI | Bronchial brushing | 2 | 1.2 | 67.0 |
| | Bronchoalveolar lavage | 18 | 11.0 | |
| | Endotracheal aspirate | 25 | 15.2 | |
| | Sputum | 62 | 1.8 | |
| | Other | 3 | 37.8 | |
| BSI | Blood | 36 | 22.0 | 22.0 |
| SSI | Abscess | 3 | 1.8 | 5.5 |
| | Wound | 6 | 3.7 | |
| IAI | Abscess | 1 | 0.6 | 1.2 |
| | Other | 1 | 0.6 | |
| UTI | Urine | 5 | 3.0 | 3.0 |
| Other | Unknown | 2 | 1.2 | 1.2 |
| Total | | 164 | 100 | |

BSI, Bloodstream infection; IAI, intra-abdominal infection; RTI, respiratory tract infection; SSI, surgical site infection; UTI, urinary tract infection.

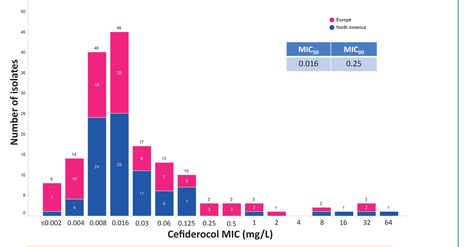
*B. cepacia complex* were 67.0 % from respiratory, 22.0 % from blood, and 5.5 % from skin.

### Table 2. Distribution of isolates tested by country

| Organism Name | Region | Country | Number of Test Strain | Ratio (%) |
|---|---|---|---|---|
| *Burkholderia cenocepacia* | Europe | France | 2 | 1.2 |
| | | Germany | 2 | 1.2 |
| | | Greece | 1 | 0.6 |
| | | Spain | 4 | 2.4 |
| | | Turkey | 3 | 1.8 |
| | North America | Canada | 8 | 4.9 |
| | | United States | 13 | 7.9 |
| *Burkholderia cepacia* | Czech Republic | | 4 | 2.4 |
| | Europe | France | 3 | 1.8 |
| | | Germany | 2 | 1.2 |
| | | Hungary | 5 | 3.0 |
| | | Italy | 13 | 7.9 |
| | | Russian Federation | 1 | 0.6 |
| | | Spain | 14 | 8.5 |
| | | Sweden | 1 | 0.6 |
| | | Turkey | 5 | 3.0 |
| | North America | Canada | 10 | 6.1 |
| | | United States | 36 | 22.0 |
| *Burkholderia dolosa* | North America | United States | 1 | 0.6 |
| *Burkholderia multivorans* | Europe | Czech Republic | 10 | 6.1 |
| | | Germany | 1 | 0.6 |
| | | Spain | 1 | 0.6 |
| | | Turkey | 2 | 1.2 |
| | | United Kingdom | 7 | 4.3 |
| | North America | Canada | 2 | 1.2 |
| | | United States | 11 | 6.7 |
| *Burkholderia vietnamensis* | North America | Canada | 1 | 0.6 |
| | | United States | 1 | 0.6 |
| Total | | | 164 | 100 |

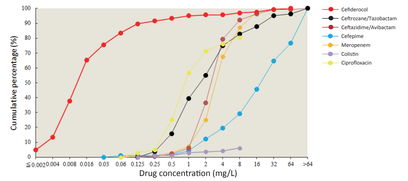
## RESULTS

### Figure 1.Cefiderocol MIC distribution against 164 *B. cepacia* complex




95.7 % of *B. cepacia complex* isolates had cefiderocol MIC values of ≤4 mg/L.

### Figure 2. Cumulative percentage of cefiderocol MIC against 164 *B. cepacia* complex.




- Cefiderocol MIC$_{90}$ values were 0.25 mg/L for *B. cepacia* complex.
- The *in vitro* activity of cefiderocol was more potent than those of comparator tested.

### Table 3. *In vitro* activity of cefiderocol against *B. cepacia* complex

| Species | Compound | MIC range | | | MIC$_{50}$ | MIC$_{90}$ |
|---|---|---|---|---|---|---|
| *Burkholderia cepacia* (94) | Cefiderocol | ≤ 0.002 | - | 16 | 0.015 | 0.12 |
| | Cefepime | 1 | - | > 64 | 32 | > 64 |
| | Ceftazidime-avibactam | 1 | - | 32 | 4 | 8 |
| | Ceftolozane-tazobactam | 0.25 | - | > 64 | 4 | 32 |
| | Ciprofloxacin | ≤ 0.12 | - | > 8 | 1 | > 8 |
| | Colistin | ≤ 0.25 | - | > 8 | > 8 | > 8 |
| | Meropenem | 0.25 | - | 16 | 4 | 8 |
| *Burkholderia cenocepacia* (33) | Cefiderocol | ≤ 0.002 | - | 0.12 | 0.015 | 0.03 |
| | Cefepime | 8 | - | > 64 | 32 | > 64 |
| | Ceftazidime-avibactam | 0.5 | - | > 64 | 4 | 8 |
| | Ceftolozane-tazobactam | 0.25 | - | > 64 | 2 | 32 |
| | Ciprofloxacin | ≤ 0.12 | - | > 8 | 1 | > 8 |
| | Colistin | 1 | - | > 8 | > 8 | > 8 |
| | Meropenem | 1 | - | 32 | 4 | 8 |
| *Burkholderia multivorans* (34) | Cefiderocol | ≤ 0.002 | - | 64 | 0.03 | 32 |
| | Cefepime | ≤ 0.06 | - | > 64 | 4 | > 64 |
| | Ceftazidime-avibactam | 0.25 | - | 16 | 2 | 8 |
| | Ceftolozane-tazobactam | 0.25 | - | > 64 | 1 | 32 |
| | Ciprofloxacin | ≤ 0.12 | - | > 8 | 2 | > 8 |
| | Colistin | ≤ 0.25 | - | > 8 | > 8 | > 8 |
| | Meropenem | 1 | - | 64 | 4 | 16 |
| *Burkholderia vietnamensis* (2) | Cefiderocol | 0.008 | - | 0.015 | N.C. | N.C. |
| | Cefepime | 0.5 | - | 4 | N.C. | N.C. |
| | Ceftazidime-avibactam | 1 | - | 4 | N.C. | N.C. |
| | Ceftolozane-tazobactam | 0.5 | - | 1 | N.C. | N.C. |
| | Ciprofloxacin | 0.5 | - | 0.5 | N.C. | N.C. |
| | Colistin | > 8 | - | > 8 | N.C. | N.C. |
| | Meropenem | 0.12 | - | 0.5 | N.C. | N.C. |
| *Burkholderia dolosa* (1) | Cefiderocol | | 1 | | N.C. | N.C. |
| | Cefepime | | 1 | | N.C. | N.C. |
| | Ceftazidime-avibactam | | 4 | | N.C. | N.C. |
| | Ceftolozane-tazobactam | | 4 | | N.C. | N.C. |
| | Ciprofloxacin | | 1 | | N.C. | N.C. |
| | Colistin | | > 8 | | N.C. | N.C. |
| | Meropenem | | 8 | | N.C. | N.C. |

### Table 4. Summary of susceptibility percentage of cefiderocol

| Organism | MIC$_{50}$/MIC$_{90}$ | MIC range | Susceptibility percent (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cefiderocol MIC ≤4 mg/L | MEM MIC ≤4 mg/L[†] | CZA MIC ≤8 mg/L[‡] | C/T MIC <4 mg/L[‡] | CIP MIC ≤0.5mg/L[‡] | CST MIC ≤2 mg/L[‡] |
| All *B. cepacia* complex (N=164) | 0.015/0.25 | 0.002-64 | 95.7 | 67.7 | 92.1 | 75.0 | 25.0 | 3.7 |
| *B. cepacia* complex (EU) (N=81) | 0.015/0.5 | 0.002-32 | 96.3 | 69.1 | 91.4 | 71.6 | 25.9 | 6.2 |
| *B. cepacia* complex (US) (N=83) | 0.015/0.12 | 0.002-64 | 95.2 | 67.1 | 68.7 | 59.0 | 15.7 | 0 |
| MEM-NS *B. cepacia* complex (N=53)* | 0.06/2 | 0.002-64 | 94.3 | 0 | 79.2 | 47.1 | 5.7 | 1.9 |

*MEM non-susceptible *B. cepacia* complex was defined strains had an MIC value of ≥8 mg/L for the meropenem.
[†]Selection criteria was used by applying CLSI breakpoint for *B. cepacia* complex.
[‡]Selection criteria was used by applying EUCAST breakpoint for *P. aeruginosa.*

- Even against meropenem non-susceptible *B. cepacia*, cefiderocol showed activity against 94.4 % of the isolates at MIC of ≤ 4 mg/L, respectively.

### Table 5. Whole genome sequencing for cefiderocol non-susceptible isolates from SIDERO-WT study.

| Strain | Origin Country | Cefiderocol MIC | MLST | β-Lactamase summary | *fiu*-like |
|---|---|---|---|---|---|
| *B. multivorans* 1557705 | United States | 32 | N.T | N.T | N.T |
| *B. multivorans* 1543270 | United Kingdom | 32 | 374 | PEN-A like partial CDS | 100% ident to WP_035955274.1 |
| *B. multivorans* 1543273 | United Kingdom | 32 | 1088 | PEN-A like partial CDS | Premature stop, 203 AA protein |
| *B. multivorans* 1484350 | United States | 32 | 117 | PEN-A like | Premature stop, 53 AA protein |
| *B. multivorans* 1543280 | United Kingdom | 8 | 803 | PEN-A like partial CDS | 99% ident to WP_012216643.1 |
| *B. multivorans* 1523723 | United States | 8 | 666 | PEN-A like | 2 nt insertion "AC" causing frameshift, 250 AA product |
| *B. cepacia* 1219352 | United States | 16 | N.T | N.T | N.T |

- Five *B. multivorans* possessed the *penA* gene, a class A β-lactamase responsible for primary resistance to β-lactam antibiotics.
- Three of the five exhibited disruptions in homologs of the catecholate siderophore receptor (*fiu*-like) genes.
- These combinations could potentially be responsible for the observed high-level cefiderocol resistance.

### Table 6.Therapeutic efficacies against mouse systemic infection model caused by *B. cepacia* SR25744.

| Test Organism (Challenge Dose: CFU/mouse) | Test and Reference Substances | ED$_{50}$* (mg/kg/dose) | 95% Confidence Interval | MIC[†] (mg/L) |
|---|---|---|---|---|
| ***B. cepacia* SR25744[‡] (1.2 × 10$^5$)** | Cefiderocol | 2.11 | 0.378 – 11.8 | 0.016 |
| | FEP | >100 | NC | 16 |
| | CZA | >100 | NC | 2 |
| | MEM/Cilastatin | 4.83 | 0.225 – 22.9 | 8 |
| | CST [‡] | >10.0 | NC | >32 |

Animal: JcI:ICR mice, male; n = 5
Infection: intraperitoneal injection
Subcutaneous administration; 1, 3, and 5 hours after infection (‡; 1 and 5 hours after infection)
*Calculated by logit analysis method from the survival rates on the seventh day after the infection. Each dose of CZA and MEPM/Cilastatin was represented as the dose of ceftazidime and MEPM, respectively. The ratio of CAZ and AVI was 4:1, and that of MEM and Cilastatin was 1:1.
[†]MIC values of MEM/Cilastatin represent the MIC values of MEPM alone.

## CONCLUSIONS

1) CFDC showed potent antimicrobial activity against *B. cepacia* complex including MEM resistant isolates.
2) WGS data indicate that combination the presence of *penA* gene and the disruptions in *fiu*-like genes likely contribute to CFDC resistance.
3) CFDC showed potent *in vivo* efficacy reflecting *in vitro* activity against *B. cepacia* in murine systemic infection model.
4) CFDC is the first β-lactam antibiotic to show potent *in vivo* activity against *B. cepacia* complex.

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