



In Vitro Susceptibility of *Burkholderia pseudomallei* Isolates to Cefiderocol and Ceftazidime/Avibactam from Odisha, India

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Abstract

Introduction and Objectives The availability of a limited arsenal of antibacterial agents effective against *Burkholderia pseudomallei*, the causative agent of melioidosis, together with sporadic reports of emergence of resistance necessitates an evaluation of in vitro activity of new antimicrobials against clinical *B. pseudomallei* isolates. Cefiderocol (CFDC), a novel siderophore cephalosporin, and ceftazidime-avibactam (CZA), a new β lactam combination agent, have shown promising results for the treatment of difficult-to-treat Gram-negative bacilli infections with limited treatment options. This study was conducted to determine the in vitro activity of CFDC and CZA against a contemporary collection of 60 *B. pseudomallei* clinical isolates.

Materials and Methods Minimum inhibitory concentrations (MIC) of CFDC and CZA were determined by broth microdilution and E-test, respectively. The performance of disk diffusion was also evaluated for CFDC.

Results All *B. pseudomallei* isolates were susceptible to CFDC and CZA with MIC range of 0.125 to 2 mg/L and 0.19 to 1 mg/L, respectively. Zone diameters for CFDC ranged from 31 to 40 mm.

Conclusion CFDC and CZA exhibited excellent in vitro activity against 60 *B. pseudomallei* isolates. Further pharmacokinetic-pharmacodynamics studies and clinical trials are needed to prove the clinical efficacy of CFDC and CZA in the treatment of melioidosis.

Keywords

- ▶ *Burkholderia pseudomallei*
- ▶ Cefiderocol
- ▶ Ceftazidime-avibactam

Introduction

Melioidosis, with myriad clinical manifestations encompassing acute, rapidly progressing septicemic infection with or without underlying focus to chronic, persistent, localized infections, often confounds initial diagnostic assessment,

and thus initiation of appropriate antimicrobial therapy. The antimicrobial therapy is biphasic with an initial intensive phase with intravenous ceftazidime or carbapenem with or without co-trimoxazole depending upon the site and extent of disease involvement. The duration of the intensive phase ranges from 2 to 8 weeks and is again determined by

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extent, site of disease along with the duration of blood culture positivity.¹ The eradication phase is aimed at preventing relapse and requires at least 3 months of treatment with oral co-trimoxazole as the first choice and amoxicillin-clavulanic acid or doxycycline as alternatives.² The limited arsenal of antibacterial agents available for the intensive phase is due to the robust intrinsic resistance mechanisms of *B. pseudomallei*. There are sporadic reports of the emergence of resistance to ceftazidime as well as carbapenems, mostly during treatment and occasional reports of de novo resistance to these drugs, from various endemic countries including India.^{3–7} Resistance to ceftazidime can be mediated by upregulation or amino acid substitution of the β -lactamase-encoding gene *penA* or the deletion of penicillin-binding protein 3 (PBP3).² Mutations in the multidrug resistance-nodulation-division efflux pump regulators often account for decreased meropenem susceptibility.⁸

Cefiderocol (CFDC) a novel siderophore cephalosporin employs an undercover trojan horse like strategy to gain access to the periplasmic space thus overcoming resistance barriers like porin loss, efflux pump in Gram-negative bacilli (GNB).⁹ Ceftazidime-avibactam (CZA) is a new β lactam combination agent. Ceftazidime coupled with non- β -lactam β -lactamase inhibitor avibactam (CZA) diminishes the availability of active site of β -lactamases for hydrolysis.¹⁰ Both CFDC and CZA have shown promise for the treatment of difficult to treat GNB infections with limited treatment options, including nonfermenting GNB (NF-GNB).^{9,10} There are only a handful of literature available regarding the activity of CFDC and CZA against clinical *B. pseudomallei* isolates, and none from India. Hence, this study was conducted to determine the in vitro activity of CFDC and CZA against a contemporary collection of *B. pseudomallei* isolates.

Method

Bacterial Isolates

A total of 60 stored clinical *B. pseudomallei* isolates (recovered from 60 patients with culture-confirmed melioidosis, from 2018–21, kept in glycerol stocks at -80°C) were revived by inoculating in tryptic soy broth, and incubation at 37°C for 5 to 7 days. Subcultures were performed on Sheep blood agar, MacConkey agar, and Ashdown agar, and plates were incubated at 37°C . Isolate identification was confirmed by conventional-polymerase chain reaction targeting a type III secretion system gene cluster.¹¹ The collection composed primarily of bloodstream (46.6%,28/60), followed by skin and soft tissue (41.6%,25/50), and respiratory (7/60,11.6%) isolates. Out of 60 patients, a favorable outcome was noted in 46 (47/60,76.6%), eight patients succumbed to the disease (8/60,13.3%), and three patients each had relapsed and left hospital against medical advice. The antimicrobial susceptibility of the isolates to ceftazidime, imipenem, meropenem, co-trimoxazole, doxycycline, and amoxicillin-clavulanic acid was determined by E-test. There was no association between drug resistance and mortality. The study was approved by the Institute Ethics Committee [T/IM-NF/Micro/21/177, dated 31.03.22].

CFDC MIC Determination

Pure powder of CFDC was obtained from Chemscene India Pvt Ltd. CFDC stock solution ($1,000\ \mu\text{g}/\text{mL}$) was prepared in normal saline, and a range of working concentrations (0.125 – $16\ \mu\text{g}/\text{mL}$) were made by serial twofold dilutions. For CFDC minimum inhibitory concentrations (MIC) determination, iron-depleted cation adjusted Muller Hinton broth (ID-CAMHB) was prepared as per Clinical & Laboratory Standards Institute (CLSI) recommendations.^{12,13} Iron-chelating agent [Chelex 100 sodium, Sigma Aldrich: C7901-25G] was added in a ratio of 1:10 to double strength CAMHB (Sigma Aldrich:90922) with continuous stirring in a magnetic stirrer for 2 hours. Calcium ($22.5\ \text{mg}/\text{L}$ as Ca^{2+}), magnesium ($11.25\ \text{mg}/\text{L}$ as Mg^{2+}), and zinc ($10\ \mu\text{M}$ as ZnSO_4) corresponding to $0.65\ \text{mg}/\text{L}$ as Zn^{2+}) were added to the ID-CAMHB to replenish the desired cations. Final concentrations of ions (iron, calcium, magnesium, and zinc) of the prepared ID-CAMHB were measured using atomic emission spectrometry for iron and zinc and inductive coupled plasma-optical emission spectrometry for calcium and magnesium. ID-CAMHB after cation reconstitution was sterilized by $0.22\ \mu\text{m}$ hydrophilic polytetrafluoroethylene syringe filter and the pH was adjusted to (7.2–7.4) with 1N hydrochloric acid. For CFDC broth microdilution (BMD), in order to achieve $100\ \mu\text{L}$ of volume in each well of the 96-well polystyrene plate, $25\ \mu\text{L}$ of reconstituted CFDC of various concentration, $25\ \mu\text{L}$ *B. pseudomallei* inoculum of concentration (5×10^5 CFU/mL), and $50\ \mu\text{L}$ of ID-CAMHB were added and incubated at $35 \pm 2^{\circ}\text{C}$ for 16 to 20 hours. MIC was interpreted as the lowest concentration of CFDC that completely inhibited visible growth of *B. pseudomallei* in the wells as detected by the unaided eye.

CZA MIC Determination

CZA E-test was performed by using CZA MIC-strips (CZA 256, Biomerieux, Diagnostics, USA) on Muller Hinton agar (MHA) as per manufacturer's instructions. In the CZA E-test strip, ceftazidime concentrations range from 0.016 to $256\ \mu\text{g}/\text{mL}$ and the concentration of avibactam is fixed at $4\ \mu\text{g}/\text{mL}$. E-test end-points were interpreted visually by the unaided eye.

CFDC Disk Diffusion

Disk diffusion (DD) was performed on unsupplemented MHA using $30\ \mu\text{g}$ Cefiderocol disk (FDC $30\ \mu\text{g}$, Liofilchem, s.r.l., Roseto Degli Abruzzi, Teramo, Italy) as per CLSI recommendations.^{12,13}

Control Strains

Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains for BMD, E-TEST, and DD.

Result Interpretation

CLSI-approved CFDC MIC clinical breakpoints for *Enterobacteriales*, *P. aeruginosa*, and *Acinetobacter* species ($\leq 4\ \text{mg}/\text{L}$ [susceptible], $8\ \text{mg}/\text{L}$ [intermediate], and $\geq 16\ \text{mg}/\text{L}$ [resistant]) were used. For CZA, MIC less than or equal to $4\ \text{mg}/\text{L}$

were regarded as susceptible, and MIC more than 8 mg/L were regarded as resistant as per CLSI clinical susceptibility breakpoints against *Enterobacteriaceae* and *P. aeruginosa*.

Results

CFDC MIC of *B. pseudomallei*

All isolates were susceptible to CFDC based on CLSI breakpoints of CFDC against *Enterobacteriales*, *P. aeruginosa*, and *Acinetobacter* species. MIC range of 60 *B. pseudomallei* isolates was 0.125 to 2 mg/L. Of the 60 isolates, 54 (54/60, 90%) were inhibited at CFDC concentration of less than or equal to 0.5 mg/L and the remaining 6 (6/60, 10%) were inhibited at 1 and 2 mg/L. Both MIC₅₀ and MIC₉₀ were 0.5 mg/L (► **Table 1**).

CZA MIC of *B. pseudomallei*

All isolates were susceptible to CZA based on CLSI breakpoints of CZA against *Enterobacteriales*, and *P. aeruginosa*. CZA MIC range of 60 *B. pseudomallei* isolates were 0.19 to 1 mg/L. Of the 60 isolates, 32 (32/60, 53.3%) were inhibited at CZA concentration of less than or equal to 0.5 mg/L and the remaining 28 (28/60, 46.6%) were inhibited at 0.75 and 1 mg/L. CZA MIC₅₀ was 0.5 mg/L (► **Table 2**). CZA E test of an isolate is depicted in ► **Fig. 1**.

CFDC Disk Diffusion

All isolates were susceptible to CFDC based on CLSI CFDC DD breakpoints of against *Pseudomonas aeruginosa*, *Acinetobacter baumannii*. Zone diameters ranged from 31 to 40 mm. Correlation between CFDC zone diameter and MIC is presented in ► **Fig. 2**. MIC ranges of 60 clinical *B. pseudomallei* isolates to ceftazidime, carbapenem (imipenem and meropenem), Co-trimoxazole, amoxicillin-clavulanic acid and doxycycline, along with CFDC and CZA, are depicted in ► **Table 3**. All isolates retained susceptibility to all the

antimicrobials tested, except two isolates which had high meropenem MIC. Those isolates were obtained from patients without meropenem treatment history and retained susceptibility to imipenem.

Discussion

Given the limited therapeutic choices during the intensive phase of melioidosis treatment, and sporadic reports of resistance to the first line antimicrobial agents, evaluation of efficacy of novel antimicrobial agents against clinical *B. pseudomallei* isolates becomes extremely justified. In our setting, two such recently launched therapeutic agents, CFDC and CZA, were found to have excellent in vitro activity against a contemporary collection of 60 clinical *B. pseudomallei* isolates. In the absence of CLSI clinical breakpoints for *B. pseudomallei*, we used the for *Enterobacteriales*, *P. aeruginosa*, and *Acinetobacter* species for MIC interpretation of *B. pseudomallei* isolates (12,13). CFDC MIC₅₀ and MIC₉₀ of 60 *B. pseudomallei* isolates in our study were 0.5 mg/L, slightly higher than MIC₉₀ values of 0.125 mg/L of 246 clinical isolates of *Burkholderia pseudomallei* from Queensland, Australia, and MIC₉₀ of 0.25 mg/L obtained from a study of 30 USAMRIID *B. pseudomallei* isolates.^{14,15} In the Australian study, four isolates (1.6%, 4/246) were categorized as nonsusceptible based on MIC more than 4 mg/L, in isolates without a prior treatment history with CFDC.¹⁴ However, those isolates were from patients with significant co-morbidities, prior exposure to multiple antibiotics including β-lactams and also had co-expression of resistance to multiple antibiotics used for melioidosis treatment.¹⁴ In our setting, though most of the *B. pseudomallei* isolates were from patients with multiple underlying risk factors and comorbidities, and two isolates had high meropenem MIC, all of them retained susceptibility to

Table 1 Cefiderocol MICs ranges of 60 *Burkholderia pseudomallei* isolates

<i>B. pseudomallei</i> (n = 60)	No. of isolates at cefiderocol MIC (mg/L) (≤4 mg/L [susceptible], 8 mg/L [intermediate], and ≥16 mg/L [resistant]) ^a							
	0.125	0.25	0.5	1	2	4	8	16
	5	23	26	4	2	0	0	0

Abbreviations: CLSI, Clinical & Laboratory Standards Institute; MICs, minimum inhibitory concentrations.

^aCLSI cefiderocol MIC clinical breakpoints for *Enterobacteriales*, *Pseudomonas aeruginosa*, and *Acinetobacter* species. MIC clinical breakpoints for *B. pseudomallei* is currently not available.

Table 2 Ceftazidime-avibactam MICs ranges of 60 *Burkholderia pseudomallei* isolates

<i>B. pseudomallei</i> (n = 60)	No. of isolates at ceftazidime-avibactam MIC (mg/L): (≤4 mg/L [susceptible], and ≥8 mg/L [resistant]) ^a						
	0.19	0.25	0.38	0.5	0.75	1	1.5
	2	3	8	19	20	8	0

Abbreviations: CLSI, Clinical & Laboratory Standards Institute; MICs, minimum inhibitory concentrations.

^aCLSI ceftazidime-avibactam MIC clinical breakpoints against *Enterobacteriaceae* and *Pseudomonas aeruginosa*. MIC clinical breakpoints for *B. pseudomallei* is currently not available.

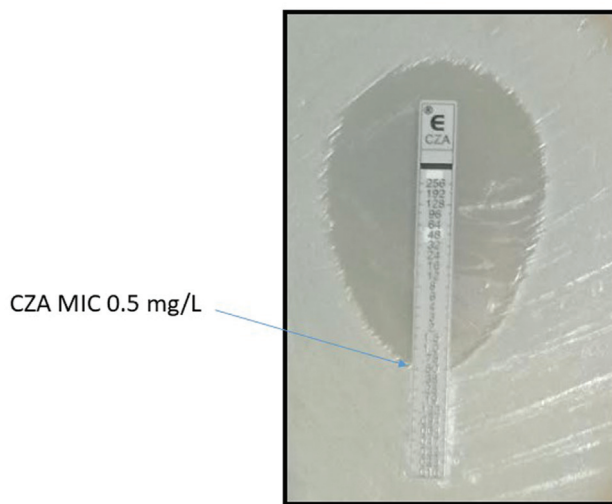


Fig. 1 Ceftazidime-avibactam E test showing MIC of 0.5 mg/L.

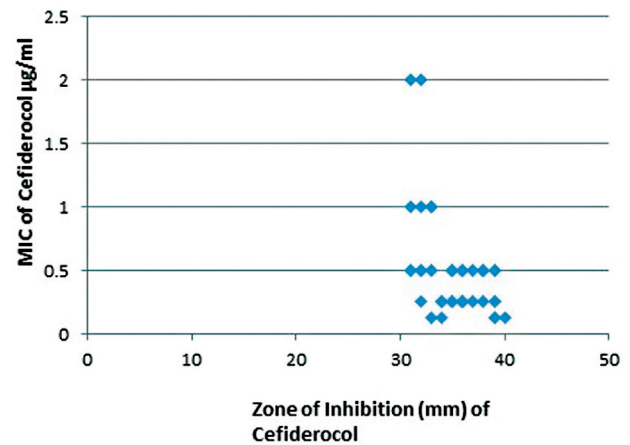


Fig. 2 Correlation of CFDC disc inhibition zone diameters and MIC values in 60 *Burkholderia pseudomallei* isolates.

Table 3 Antimicrobial susceptibility pattern of 60 *Burkholderia pseudomallei* isolates

Serial no.	Antimicrobials and MIC breakpoints	MIC range (mg/L)	% susceptibility
1	Cefiderocol (≤ 4 mg/L [S], 8 mg/L [I], ≥ 16 mg/L [R])	0.125–2	100
2	Ceftazidime-avibactam (≤ 4 mg/L [S], ≥ 8 mg/L [R])	0.19–1	100
3	Ceftazidime (≤ 8 mg/L [S], 16 mg/L [I], ≥ 32 mg/L [R])	0.5–4	100
4	Imipenem (≤ 4 mg/L [S], 8 mg/L [I], ≥ 16 mg/L [R])	0.125–3	100
5	Meropenem (≤ 4 mg/L [S], 8 mg/L [I], ≥ 16 mg/L [R])	0.25–32	96.6
6	Co-trimoxazole ($\leq 2/38$ mg/L [S] $\geq 4/76$ mg/L [R])	0.032–2	100
7	Amoxicillin-clavulanic acid ($\leq 8/4$ mg/L [S], 16/8 mg/L [I], $\geq 32/16$ mg/L [R])	1–4	100
8	Doxycycline (≤ 4 mg/L [S], 8 mg/L [I], ≥ 16 mg/L [R])	0.5–2	100

Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

CFDC. BMD for CFDC is technically demanding, and requires careful monitoring at various steps including cation depletion, cation replenishment, and final pH adjustment.^{12,13} The agreement and error rates of CFDC DD with BMD depend upon manufacturers of CFDC disc and CA-MHB as well as Genera of GNB.^{16–18} In our study, overall zone diameters ranged from 31 to 40 mm. For *B. pseudomallei* isolates with MIC more than 0.5 mg/L, zone diameters did not exceed 33 mm. In the study by Burnard et al, for *B. pseudomallei* isolates with MICs between 1 and 4 mg/L, zone diameters ranged between 35 and 25 mm.¹⁴ Relationship between CFDC zone diameter and MIC values can be better ascertained by including resistant isolates and isolates with wider MIC distribution.

In this study, CZA MIC range of 60 clinical *B. pseudomallei* isolates was 0.19 to 1 mg/L. In the study from Thailand by Sribenjalux et al, CZA MIC₅₀ of 28 clinical *B. pseudomallei* isolates was 0.75 mg/L.¹⁹ Ceftazidime resistance in clinical *B. pseudomallei* isolates remains low, and there is a chance of underestimation of ceftazidime resistance during therapy. A recent study concluded that ceftazidime-resistant isolates with underlying PBP3 deletion fail to grow on routine laboratory media unless supplemented with glycerol and thus during or after ceftazidime therapy, samples can be plated onto glycerol containing media such as Ashdown to promote the growth of ceftazidime-resistant variants.⁴ In a study by Chirakul et al, ceftazidime resistance in a clinical *B. pseudomallei* isolate had been shown to be completely

reversed in vitro by addition of avibactam.²⁰ In this study, CZA MIC of 60 clinical *B. pseudomallei* isolates were two to fourfold lower than that of ceftazidime alone. These in vitro studies including ours show a promising role of CZA in therapy of ceftazidime-resistant *B. pseudomallei* as a carbapenem sparer agent.^{19,20}

This study, conducted on 60 isolates from a single tertiary center healthcare facility, provides the in vitro activity of cefiderocol and CZA, for the first time for *B. pseudomallei* isolates from India. Further pharmacokinetic-pharmacodynamics studies and clinical trials are needed to prove clinical efficacy of cefiderocol and CZA in the treatment of melioidosis.

Funding

None.

Conflict of Interest

None declared.

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