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ORIGINAL RESEARCH

Analysis of In Vitro Activity of Cefiderocol Against Carbapenem-Resistant Gram-Negative Bacilli by Broth Microdilution and Disk Diffusion Method: A Single-Center Study in Odisha, India

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Introduction: Cefiderocol (CFDC), a novel semi-synthetic siderophore cephalosporin has been developed to combat the menace of infections caused by carbapenem-resistant Gram-negative bacilli (CR-GNB) including Carbapenem-resistant *Enterobacterales* (CRE) and Carbapenem-resistant Nonfermenting Gram-negative bacilli (CR-NFGNB).

Methods: We determined the in vitro activity of CFDC against a contemporary collection of 503 CR-GNB isolates by the reference broth microdilution method (BMD) using Iron depleted cation adjusted Mueller-Hinton broth (ID-CAMHB). Performance of CFDC disk diffusion (DD) was evaluated against the reference BMD, as an alternative convenient testing method. Molecular characterization of carbapenemase in CR-GNB was performed by PCR targeting $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48like alleles}}$, bla_{KPC}, bla_{IMP}, and, bla_{VIM}. Minimum inhibitory concentration (MIC) distribution of CFDC in CR-GNB harbouring different carbapenemase enzymes was also analyzed.

Results: In our study, 81.7% (411/503) of CR-GNB isolates [81.3%, (278/342) CRE and 82.6% (133/161) CR-NFGNB] were susceptible to CFDC ($p>0.05$). Categorical agreement (CA) of DD ranged from 79.8% to 87.5%, Minor error (mE) ranged from 0 to 14%, Major error (ME) ranged from 0 to 3.5%, and Very Major error (VME) ranged from 0 to 12.5% with variations by species tested. Overall CFDC MIC50 and MIC90 values of CR-GNB isolates without any carbapenemase genes were higher as compared to those with the presence of carbapenemase genes (4 µg/mL and 128 µg/mL versus 2 µg/mL and 16 µg/mL respectively).

Discussion: CFDC is not yet available for clinical use in India. Hence, multicentric studies are the need of the hour in India for standardization of CFDC susceptibility using disks and CAMHB from different manufacturers as well as understanding mechanisms of high MIC values.

Keywords: Cefiderocol, carbapenem-resistant Gram negative bacilli, broth microdilution, carbapenemases, disk diffusion, minimum inhibitory concentration

Introduction

The increasing prevalence of carbapenem-resistant Gram-negative bacilli (CR-GNB) is regarded as one of the greatest public health concerns across the globe with several CR-GNB, e.g., Carbapenem-resistant *Enterobacterales* (CRE), Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and Carbapenem-resistant *Acinetobacter baumannii* (CRAB) being regarded as Priority I critical pathogens by the World Health Organization (WHO) due to existence of multiple resistance mechanisms and lack of effective antimicrobial agents against them.[1]

Cefiderocol (CFDC), a catechol substituted siderophore cephalosporin, has broad-spectrum activity against CR-GNB, including CRE, CRPA, and CRAB.[2] The siderophore moiety of CFDC employs trojan horse like strategy allowing the entry of antimicrobial into the periplasmic space to reach the target site (PBPs) by active transport. CFDC displays structural stability to hydrolysis by both serine as well as various Metallo-β -lactamases.[2] CFDC has completed Phase III clinical trial and is approved by the United States Food and Drug Administration (USFDA) for the

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treatment of complicated urinary tract infections (cUTIs), including pyelonephritis, Hospital-acquired pneumonia (HAP) or Ventilator-associated pneumonia (VAP), caused by designated susceptible GNB in adults and by European Medicines Agency (EMA) for the treatment of infections caused by aerobic GNB in adults with limited treatment options.[3,4]

Clinical and Laboratory Standards Institute (CLSI) had recommended broth microdilution (BMD) with minimum inhibitory concentration (MIC) determination as the only method for CFDC susceptibility testing in 2019 which requires iron-depleted culture media and subsequent cation adjustments to mimic the in vivo human plasma iron concentration.[5] In the year 2020, CLSI also approved the disk diffusion (DD) method as the second method for the determination of CFDC susceptibility.[6] In contrast to BMD, DD does not require iron depletion of standard Mueller-Hinton Agar (MHA) as iron is present in a bound state in the agar.[6] Few studies have evaluated DD as an alternative suitable method to BMD for the determination of CFDC susceptibility.[7,8] Moreover, there is no published literature regarding CFDC susceptibility from India. Hence, the present study was designed to determine CFDC susceptibility against a contemporary collection of CRE, CRPA, and CRAB isolates by BMD and DD. Simultaneously performance of the DD was also evaluated, taking BMD as the reference method. The MIC distribution of CFDC against CR-GNBs producing specific types of carbapenemase enzymes was analyzed.

Methods

Study Settings and Bacterial Isolates

This observational, cross-sectional study was conducted on 503 clinically significant, consecutive, non-duplicate CRE, CRPA, and CRAB isolates recovered from patients receiving medical care at a tertiary care, teaching, and referral hospital in Odisha. The isolates were collected prospectively for 2 years (Jan 2020–Dec 2021). GNB isolates testing resistant to any one of the Carbapenem group of antimicrobials (Ertapenem [only for CRE], Meropenem, Imipenem, Doripenem) were labelled as CR-GNB.[9] CFDC susceptibility testing of the CR-GNB isolates was performed by both reference BMD as well as DD. The study was approved by the institutional ethics committee of All India Institute of Medical Sciences [AIIMS], Bhubaneswar, Odisha (Ref Number: IEC/AIIMS BBSR/PG-Thesis/2019-20/100 dated 14th December 2019).

MIC Determination, DD, and Interpretation

CFDC pure powder (CS-0016, CAS No: 122520S-94-5) was obtained from Chemscene India Pvt Ltd. CFDC stock solution (1000 µg/mL) was prepared in normal saline, and desired concentrations of working solutions (0.25–128 µg/mL) were made by twofold serial dilutions using normal saline as a diluent as per CLSI recommendations.[5,6] Iron depleted Cation Adjusted Muller Hinton Broth (ID-CAMHB) was prepared as per CLSI recommendations.[5,6] Briefly, 1g of iron-chelating agent Chelex® 100 sodium (Sigma Aldrich: C7901-25G) was added to 10 mL of 2X CAMHB (Sigma Aldrich:90922). The solution was continuously stirred with the help of a magnetic stirrer for 2 hrs at room temperature. Calcium (22.5 mg/L as Ca^{2+}), magnesium (11.25 mg/L as Mg^{2+}) and zinc (10 µM as ZnSO4) corresponding to 0.65 mg/L as Zn^{2+}) were added to replenish the desired cations. The final concentrations of ions (iron, calcium, magnesium, and zinc) of the ID-CAMHB media were measured using atomic emission spectrometry (AAS) for iron and zinc and Inductive coupled plasma-optical emission spectrometry (ICP-OES) for calcium and magnesium. IDCAMHB after cation reconstitution was sterilized by 0.22µm hydrophilic polytetrafluoroethylene (PTFE) syringe filter and the final pH was adjusted to (7.2–7.4) with 1N hydrochloric acid. For BMD, to achieve 100 µL of volume in each well of the U bottom 96-well polystyrene plate (Tarson Micro Test Plate), 25 µL of drug, 25 µL inoculum of concentration (5×10^5 CFU/mL), and 50 µL of ID-CAMHB were added and incubated at 35 ± 2°C for 16 to 20 hours. MIC was read as the lowest concentration of CFDC that completely inhibited the growth of the organism in the wells as detected by the unaided eye. Trailing (multiple wells of tiny or faint growth relative to the growth control) was ignored while reading MIC. MIC_{50} and MIC_{90} values were calculated as per the standard method. DD was performed by using 30µg disk (FDC 30 µg, Liofilchem, s.r.l., Roseto Degli Abruzzi, Teramo, Italy) on unsupplemented standard Mueller-Hinton agar (MHA) (M1084 Hi-media Pvt Ltd, Mumbai, India). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (CFDC MIC range:

0.06–0.5µg/mL for both) were used as control strains for BMD and DD. CLSI breakpoints were used for the analysis of CFDC MICs and zone diameter.[5,6]

Agreement and Error Analysis

CFDC DD results were compared with MIC values for agreement and analysis of errors. CA was calculated as the percentage of isolates with results in the same category as the reference method, taking the total number of isolates tested as the denominator. Minor error (mE) was calculated as the percentage of isolates susceptible to CFDC by reference BMD method but in the intermediate category by the DD method or vice versa taking the total number of isolates tested as the denominator. Major error (ME) was calculated as the percentage of isolates showing MIC in the susceptible range by the reference BMD method and in the resistant range by DD method (false resistance by DD), taking total number of susceptible isolates by reference BMD method as the denominator. Very major error (VME) was calculated as the percentage of isolates showing MIC in the resistant range by reference BMD method and in the susceptible range by DD method (false susceptible by DD), taking total number of resistant isolates by BMD method as the denominator.

PCR for Carbapenemase Genes

Conventional uniplex PCR for five carbapenemase encoding genes ($bla_{\text{NDM-1}}$, $bla_{OXA-48\text{like alleles}}$, bla_{IMP}, bla_{VIM}, and bla_{KPC}) were performed in 319 CR-GNB isolates. Standard published primers for five major carbapenemase encoding genes were used.[10] CFDC MIC values of CR-GNB were stratified by the type of carbapenemase enzymes produced. *Klebsiella pneumoniae* ATCC BAA- 2146 and ATCC BAA-1705 were used as quality control strains for bla_{NDM} and bla_{KPC} respectively.

Results

Out of 503 CR-GNB isolates included in the present study, majority were CRE (342/503,68%) followed by CRAB (137/503, 27.2%) and CRPA (24/503,4.8%). Overall, *E. coli* was the most common isolate (183/503,36.4%) followed by *K. pneumoniae* (152/503,30.2%), *A. baumannii* complex (137/503, 27.2%), *P. aeruginosa* (24/503, 4.8%) and *E. cloacae* complex (7/503, 1.4%). Carbapenem-resistant *E.coli* isolates were recovered predominantly from urine (89/183, 48.6%) followed by blood samples (39/183, 21.3%) whereas Carbapenem-resistant *K.pneumoniae* were recovered predominantly from blood (68/152, 44.7%) followed by respiratory samples (39/152, 25.6%). The sample distribution of different genera of CR-GNB is depicted in Table 1.

CFDC MIC Distribution of CR-GNB Isolates

CFDC MIC levels were highly variable depending on the CR- GNB species. The details of MIC distribution among 503 CR-GNB isolates are depicted in Table 2. 411/503 (81.7%) of CR-GNB isolates had CFDC MIC in the susceptible range, 48/503 (9.5%) had MICs in the intermediate range and 44 44/503 (8.7%) had MICs in the resistant

Table 1 Sample-Wise Distribution of Different Genera of Carbapenem-Resistant GNB (CR-GNB)(n=503)

Specimen	*E. coli* (n = 183)	*K. pneumoniae* (n = 152)	*E. cloacae* Complex (n = 7)	*A.baumannii* Complex (n = 137)	*P. aeruginosa* (n = 24)
Blood (n = 170)	39	68	5	57	1
Urine (n = 124)	89	16	0	12	7
Respiratory (n = 123)	26	39	1	51	6
Pus and exudates (n = 54)	17	18	1	9	9
Fluid from sterile body sites (n = 32)	12	11	0	8	1

Abbreviation: n, Total number of isolates *tested.*

Table 2 CFDC MIC Distribution Against Different Genera of CR-GNB (n=503)

CR-GNB Genera	CFDC MIC in μg/mL														
	<0.25	0.25	0.5	1	2	4	8	16	32	64	128	>128	Range	MIC50	MIC 90
E. coli (n = 183)	18	0	19	25	65	17	16	4	6	2	4	7	<0.25->128	2	32
K. pneumoniae (n = 152)	4	5	30	27	36	26	14	6	1	0	0	3	<0.25->128	2	8
A. baumannii complex (n = 137)	5	6	19	20	19	43	17	2	0	0	0	6	<0.25->128	2	8
P. aeruginosa (n = 24)	1	2	6	5	5	2	1	1	1	0	0	0	<0.25–32	1	8
E. cloacae complex (n = 7)	0	0	1	1	3	1	0	0	1	0	0	0	0.5–32	2	32
Total	28	13	75	78	128	89	48	13	9	2	4	16			

Abbreviations: MIC, Minimum inhibitory concentration; MIC_{50}, Minimum inhibitory concentration (MIC)50; MIC_{90}, Minimum inhibitory concentration (MIC)90; n, Total number of isolates tested.

category. At a susceptible breakpoint level of ≤ 4μg/mL, CFDC inhibited the growth of a higher percentage of NF-GNB (133/161, 82.6%) than *Enterobacterales* (278/342, 81. 3%), though the difference was not statistically significant (p = 0.69). Overall the most susceptible isolate was *P. aeruginosa* (21/24, 87.5%) and the least susceptible isolate was *E. coli* (144/183, 78.7%). Among CFDC susceptible *Enterobacterales, K. pneumoniae* was most susceptible (128/152, 84.2%, followed by *E. coli* (144/183, 78.7%) and among CFDC susceptible NF-GNB, *P. aeruginosa* (21/24, 87.5%) was more susceptible than *A. baumannii* complex (112/137, 81.7%). Out of the total 48 CR-GNB isolates in CFDC intermediate category, maximum isolates belonged to NF-GNB (18/161,11.2%) compared to *Enterobacterales* (30/342,8.8%). Out of a total of 44 CFDC-resistant CR-GNB, *E. coli* (23/44, 52.3%) was the highest in number followed by *K. pneumoniae* (10/44, 22.7%) and *A. baumannii* complex (8/44, 18.2%), whereas only (2/44, 4.5%) isolates of CRPA were resistant to CFDC. Overall CRE and CR-NFGNB had similar MIC_{50} and MIC_{90} values ie 2μg/mL and 8 μg/mL respectively. However, on genus-wise stratification of CRE, *E. coli* had MIC_{90} value of 32 μg/mL. MIC_{50} value *P. aeruginosa* (1 μg/mL) was lower than *Acinetobacter baumannii* complex (2 μg/mL).

CFDC DD of CR-GNB Isolates

In the present study, out of the 503 CR-GNB isolates tested by DD, 408 isolates (408/503, 81.1%) were susceptible, 51 isolates (51/503, 10.2%), were intermediate and 44 isolates (44/503, (8.7%) were resistant. CFDC susceptibility of NFGNB and CRE were (144/161,89.4%) and (264/342, 77.2%) respectively by DD. *E.* complex had 100% susceptibility to CFDC by DD, but the number of isolates were very less (7). On excluding *E.* complex, overall, CRAB (123/137, 89.8%) isolates were the most susceptible and CR *E. coli* (127/183, 69.4%) were the least susceptible to CFDC by DD. Across different genera which were resistant to CFDC, the highest number of resistant isolates belonged to *E. coli* (27/44, 61.4%) followed by *K. pneumoniae* (10/44, 22.7%) and *A. baumannii* complex (6/44, 13.6%). Only 1 isolate out of 24 CRPA and none of 7 *E. cloacae* complex was resistant to CFDC by DD. The distribution of CFDC MICs to zone diameters for *Enterobacterales, A. baumannii* complex, and *P. aeruginosa* are depicted in Figure 1.

Agreement and Error Analysis of CFDC BMD and DD

Agreement analysis of CFDC DD was done using BMD as the reference method. Categorical agreement (CA), mEs, MEs, and VMEs were assessed according to standard definitions. 90% of CA, 10% of mE, and 3% of ME and VME were considered acceptable based on CLSI guidelines. Two hundred eighty isolates of CRE (146 *E. coli*, 128 *K. pneumoniae*, and 6 *E. cloacae* complex) and 138 isolates of CR-NFGNB (117 *A. baumannii* complex, 21 *P. aeruginosa*) were in CA by both the methods. Collectively, CA ranged from 79.8% to 87.5%, mE ranged from 0 to 14%, ME ranged from 0 to

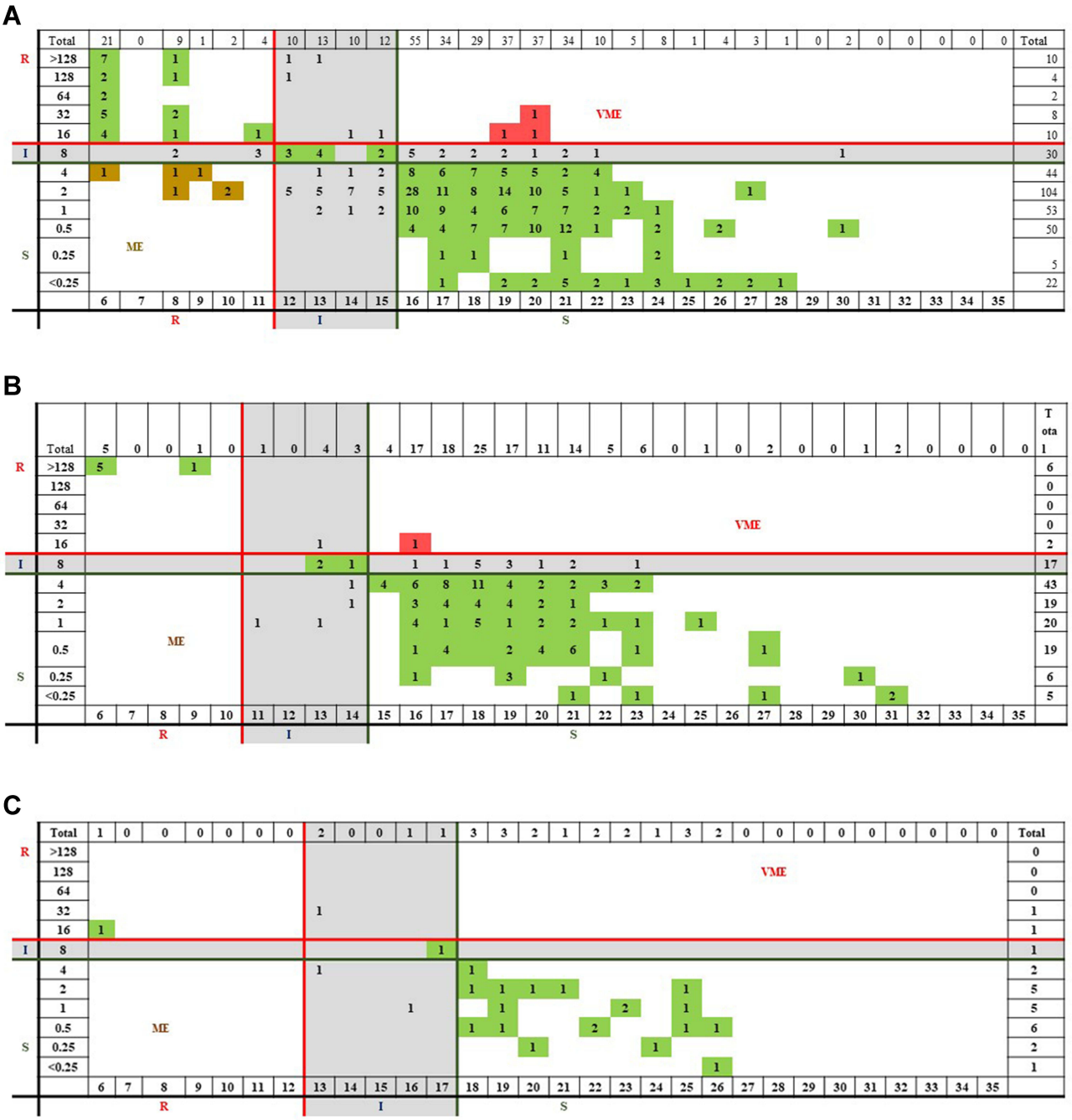

Figure 1 Distribution of CFDC MICs to zone diameters (**A**) for *Enterobacterales*, (**B**) for *A. baumannii* complex, (**C**), for *P. aeruginosa)*. BMD MICs (µg/mL) are on the X axis, and zone diameters in (mm) are on the Y axis. The isolates highlighted in Green are in Categorical agreement (CA) by both the methods. Isolates showing VME are highlighted in red and ME are highlighted in brown color. Areas highlighted with grey contain isolates of Intermediate Category Isolates above (For BMD) and left (For DD) to Red bar are RESISTANT isolates. Isolates below (For BMD) and right (For DD) to Green bar are SUSCEPTIBLE isolates.

3.5%, and VME ranged from 0 to 12.5% (excluding *E. cloacae* complex), with variations by species tested. The number of isolates of *E. cloacae* complex was too low to be considered in the analysis. The CA of DD was higher in CR-NFGNB (85.7%) than CRE (81.9%). CA was lowest for CR *E. coli* (79.8%) and highest for CRPA (87.5%). However, for all the genera, CA of DD was below CLSI acceptable range (90%). mE of DD ranged from 0 to 13.6% and was almost similar for both CRE (12.6%) and CR-NFGNB (12.4%). The mE rate of DD for CRPA was 8.3% and in *E. cloacae* complex no mE was found. For all other organisms, the mE was beyond CLSI acceptable limit ie 10%. ME of DD ranged from 0–

3.5% and was within CLSI acceptable range (<3%) for all genera, except for CR *E. coli* where ME was 3.5%. Six isolates of CRE showed ME, there were no ME in cases of CR-NFGNB. In contrast to mE, and ME, VME was higher in CR-NFGNB (10%) compared to CRE (8.8%). VME was lowest for CR *E. coli* (4.3%) Overall, VME was beyond CLSI acceptable range (<3%) for all CR-GNB, except for CRPA where there was no VME. Analysis of CFDC susceptibility results by BMD and DD, Agreement, and Error rates are summarised in Table 3.

Genotypic Profile of Carbapenemases

Out of a total of 503 CR-GNB, genotypic analysis for five major carbapenemase encoding genes ($bla_{\text{NDM-1}}$, $bla_{\text{OXA-48like allele}}$, bla_{IMP}, bla_{VIM}, and bla_{KPC}) could be performed in 319 isolates [201 CRE (85 *E. coli*, 110 *K.pneumoniae*, 6 *E. cloacae* complex), 103 CRAB and 15 CRPA]. Out of 319 isolates tested, 236 (236/319, 74%) CR-GNB isolates harbored one or more carbapenemase genes whereas 83(83/319, 26%) of CR-GNB isolates did not have any of the five tested carbapenemase genes. Among the carbapenemase producers, the highest number of isolates were NDM-1 producers (132/236, 55.9%) followed by combined NDM-1 and OXA48-like allele co-producers (76/236, 32.2%). None of the CR-GNB isolates harbored bla_{KPC}, bla_{IMP}, bla_{VIM}. The majority of CR *E. coli* (60/85, 70.6%) and CRPA (8/15, 53.3%) were NDM-1 producers. However, 44.5% (49/110) of CRKP and 42.8% (3/7) of *E.cloacae* complex isolates harbored a combination of NDM-1 and OXA 48-like allele. In the majority of CRAB (47/103,71.2%) isolates, none of the five carbapenemase genes were detected.

CFDC Susceptibility by types of Carbapenemase

Among CRE, (22/27, 81.5%) of isolates harbouring $bla_{\text{OXA 48-like alleles}}$ were susceptible to CFDC, and (66/96, 68.7%) of isolates harbouring $bla_{\text{NDM-1}}$ retained CFDC susceptibility. CRE isolates without the presence of any of the five carbapenemase genes had the least susceptibility (6/11, 54.5%) to CFDC. In case of CRAB, out of a total of 66 isolates that were not harboring any of the five carbapenemase genes, 47 (47/66,71.2%) isolates retained susceptibility to CFDC whereas out of the 37 isolates harboring one or more carbapenemase genes, 31 isolates (31/37,83.7%) were susceptible. On analysis of CFDC susceptibility based on the presence of a type of carbapenemase gene, CFDC susceptibility of CRAB isolates harboring $bla_{\text{NDM-1}}$ was 85.7% (24/28) and the percentage susceptibility of isolates harboring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA 48-like allele}}$ was 75% (6/8). In case of CRPA, out of a total of 6 isolates that were not harboring any of the five carbapenemase genes, all (6/6100%) retained susceptibility to CFDC whereas out of the 9 isolates harboring one or more carbapenemase genes, 7 isolates were susceptible (7/9,77.8%). CFDC susceptibility of CRPA isolates harboring $bla_{\text{NDM-1}}$ was 75%(6/8) and the percentage susceptibility of isolates harboring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA 48-like allelle}}$ was 100% (1/1). In cases of CRE isolates, out of a total of 190 isolates harboring any one or more carbapenemase gene/s 138 (138/190, 72.6%) isolates retained the susceptibility to CFDC whereas out of a total of 11 isolates that were not harbouring any of

Table 3 Agreement and Error Rates of DD, Taking BMD as the Reference Method

Organisms	No. of Isolates	CA [no.(%)]	mE [no.(%)]	ME [no. (%)]	VME [no.(%)]
Enterobacterales	342	280(81.9)	43(12.6)	6(2.2)	3(8.8)
E. coli	183	146(79.8)	25(13.6)	5(3.5)	1(4.3)
K. pneumoniae	152	128(84.2)	18(11.8)	1(0.8)	1(10)
E.cloacae complex	7	6(85.7)	0(0)	0(0)	1(100)
Non-glucose-fermenting GNB	161	138(85.7)	20(12.4)	0(0)	1(10)
A. baumannii complex	137	117 (85.4)	18(13.1)	0(0)	1(12.5)
P. aeruginosa	24	21(87.5)	2(8.3)	0(0)	0(0)
Total	503	418(83.1)	63(12.5)	6(1.45)	4(9.1)

Abbreviations: CA, Categorical agreement; mE, Minor error; ME, Major error; VME, Very Major error.

the five carbapenemase genes, 6 isolates were susceptible to CFDC (6/11,54.5%). CFDC susceptibility of CRE isolates harbouring $bla_{\text{NDM-1}}$ was 68.7% (66/96), percentage susceptibility of isolates harbouring $bla_{\text{OXA 48-like alleles}}$ was 81.5% (22/27) and percentage susceptibility of isolates co-possessing $bla_{\text{NDM-1}}$ and $bla_{\text{OXA 48-like alleles}}$ was 74.6% (50/67). In case of CFDC resistant CRE isolates (MIC ≥16 µg/mL), regardless of the species, isolates harbouring $bla_{\text{NDM-1}}$ had a higher MIC distribution range, compared to CRE isolates co-producing $bla_{\text{NDM-1}}$ and $bla_{\text{OXA 48-like alleles}}$ or $bla_{\text{OXA 48-like alleles}}$ alone. Furthermore, on analysing MIC_{50} and MIC_{90} values of specific groups of carbapenemase producers and carbapenemase non- producers it was found that MIC_{90} of Non -carbapenemase-producing CRE (Non-CP-CRE) (>128µg/mL) was 8-fold higher than carbapenemase-producing CRE (CP-CRE) (16µg/mL). Similarly, among CR-NFGNB, both MIC_{50} and MIC_{90} values of isolates (4 µg/mL,16µg/mL respectively) without any of the tested carbapenemase genes were two folds higher than isolates harbouring one or more carbapenemase encoding genes (2 µg/mL and 8 µg/mL respectively). [Table 4]

Table 4 Distribution of MIC Across Different Carbapenemase Genes and Specific Groups of CR-GNB (n=319)

	Cefiderocol MIC											**%Susceptible**		**MIC Values (µg/mL)**		
Enterobacterales (n=201)	**>128**	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**<0.25**	**% (No.)**	**MIC_{50}**	**MIC_{90}**	**Total**
Carbapenemase-producing –CRE													72.6 (138)	2	16	190
Non-Carbapenemase producing –CRE													54.5(6)	2	>128	11
MIC by type of carbapenemase in CRE																
NDM-1	5	2	2	3	5	13	15	31	10	7	2	1	68.7(66)			96
NDM-1 and OXA48-like allele	1	1	0	2	3	10	15	25	6	3	1	0	74.6(50)			67
OXA 48-like allele	0	0	0	0	0	5	5	9	6	2	0	0	81.5(22)			27
No genes detected	2	1	0	1	0	1	1	1	1	2	0	1	54.5(6)			11
Non-glucose fermenting GNB (n=118)																
Carbapenemase-producing – NFGNB													82.6(38)	2	8	46
Non-Carbapenemase producing – NFGNB													73.6(53)	4	16	72
MIC by type of carbapenemase in CR-NFGNB																
Acinetobacter baumannii complex																
NDM-1	0	0	0	0	0	4	8	6	3	6	1	0	85.7(24)			28
NDM-1 and OXA48-like allele	0	0	0	0	0	2	2	4	0	0	0	0	75(6)			8
OXA 48-like allele	0	0	0	0	0	0	0	0	0	0	1	0	100(1)			1
No genes detected	6	0	0	0	2	11	27	6	8	2	3	1	71.2(47)			66
P. aeruginosa																

(*Continued*)

Table 4 (Continued).

	Cefiderocol MIC											%Susceptible		MIC Values (µg/mL)		
Enterobacterales (n=201)	**>128**	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**<0.25**	**% (No.)**	**MIC_{50}**	**MIC_{90}**	**Total**
NDM-1	0	0	0	0	1	1	0	2	2	1	1	0	75(6)			8
NDM-1 and OXA48-like allele	0	0	0	0	0	0	0	1	0	0	0	0	100(1)			1
OXA 48 like allele	0	0	0	0	0	0	0	0	0	0	0	0	0(0)			0
No genes detected	0	0	0	0	0	0	2	1	0	2	1	0	100(6)			6

Abbreviations: CRE, Carbapenem-resistant *Enterobacterales*; NFGNB, Non-glucose fermenting GNB; NDM, New Delhi-metallo β-lactamases; OXA 48, Oxacillinases 48.

Discussion

The present study demonstrates the performance of the CFDC susceptibility testing against CR-GNB isolates in an Indian tertiary care setting. In our study, 81.7% of CR-GNB isolates had CFDC MIC in the susceptible range. CFDC susceptibility of CRE isolates was 81.3% and of CR-NFGNB was 82.6%. In vitro susceptibility data of CFDC in three consecutive multinational surveillance studies (SIDERO-WT studies), covering over 28,000 Gram-negative bacteria [9205 clinical isolates in 2014–2015, 8954 in 2015–2016, and 10,470 in 2016–2017] had found CFDC susceptibility in >99% of isolates during each testing period.[11–13] The SIDERO-WT studies included both carbapenem susceptible and non-susceptible isolates, whereas our study focussed only on CR-GNB isolates. Naas et al and Longshaw et al also investigated the in vitro activity of CFDC against GNB isolates from France and Europe respectively.[14,15] In the study by Naas et al, overall, 99.0% of *Enterobacterales* and 99.7% of NF-GNB were CFDC susceptible.[14] In the study by Longshaw et al, 81.6% of *Enterobacterales*, and 98.3% of *P. aeruginosa* were CFDC susceptible.[15] Candel et al studied in vitro activity of CFDC in 11 European countries and the susceptibility rate of Carbapenem-resistant *E. coli, Klebsiella* spp., *P. aeruginosa, Acinetobacter* spp. to CFDC was 77.8%, 69.2%, 97.5% and 90.7% respectively.[16] The CFDC susceptibility rate of CR-GNB isolates in our study is less than SIDERO-WT studies and matches the findings of Longshaw et al and Candel et al. These regional differences in CFDC susceptibility need further analysis.

In our study, among CRE isolates 85.7% (6/7) of *Enterobacter cloacae* complex, 84.2% (128/152) of *K. pneumoniae*, and 78.7% (144/183) of *E. coli* retained susceptibility to CFDC. However, in the studies by Longshaw et al and Candel et al *Klebsiella* spp. was the least susceptible isolate (82.8% and 69.2% respectively).[15,16] In our study among CR-NFGNB, 87.5% (21/24) of *P. aeruginosa* and 81.7% (112/137) of *A. baumannii* complex were susceptible to CFDC. The percentage susceptibility rate of CRAB and CRPA isolates in our study is nearly similar to the findings of the study by Mushtaq et al in which 88.9% CRAB and 86.5% CRPA were susceptible to CFDC.[17] In our study, out of 44 CFDC-resistant CR-GNB, *E. coli* (52.3%,23/44) was the highest in number followed by *K. pneumoniae* (22.7%,10/44). Similar to our findings, Iregui et al had described higher CFDC MICs in endemic *E. coli* and *K. pneumoniae* isolates.[18] However, in the study by Ghebremedhin et al, the prevalence of CFDC resistance was reported to be more in CR-NFGNB (23.1% in CRAB and 18.9% in CRPA).[19]

In the present study CA of DD ranged from 79.8% to 87.5%, mE ranged from 0 to 14%,ME ranged from 0 to 3.5%, and VME ranged from 0 to 12.5% with variations by species tested. ME rates of all CR-GNB isolates were within the acceptable range of CLSI (3%) except for *E.coli* in which ME (3.5%) was beyond the acceptable range of CLSI. ME of CRE was 2.2% and no ME was observed in case of CR-NFGNB isolates. In our study, VME rates of all CR-GNB isolates were beyond the acceptable range of CLSI (3%) except for CRPA (no VME was found). Morris et al had compared the susceptibility results of CFDC DD against BMD in 58 CRE and 50 CRNF-GNB isolates using FDA-approved Hardy Disk and research-use-only (RUO) MASTDISCS and interpreted results by FDA, EUCAST, and investigational CLSI breakpoints (BPs).[20] Agreement analysis and error analysis in their study varied between different

disks, different species, and using different interpretative criteria. In a recent study by Bonnin et al, DD had 77% CA compared to BMD.[21] The overall CA (83.1%) of DD in our study using Liofilchem RUO disk was lower than the overall CA (89%) reported by Morris et al but higher than the CA (77%) reported by Bonnin et al[20,21] Our study used CAMHB and CFDC disk of single manufacturers. Further large-scale studies using disks and CA-MHB from different manufacturers are warranted to evaluate the utility of DD as an alternative to BMD for the determination of CFDC susceptibility.

In our study, overall CFDC MIC_{90} values of 503 CR-GNB isolates were 8 µg/mL. Among different genera of CRE, *E. coli* had the highest MIC_{90} value ie 32µg/mL, and MIC_{90} values of CRAB and CRPA were 8 µg/mL. These findings are nearly similar to the findings of the study by Hackel et al, which reported CFDC MIC_{90} values of CRE:4µg/mL, MDR *A. baumannii*:8µg/mL, MDR *P. aeruginosa*:1µg/mL, whereas the MIC_{90} values of isolates in the present study are higher than those described in SIDERO-WT studies.[11–13,20] In SIDERO-WT studies, the MIC_{90} value of CFDC ranged from 0.25 to 1 µg/mL for *Enterobacterales* and 0.03 to 1 µg/mL for *P. aeruginosa* and 1 to 4 µg/mL for *A. baumannii* over three years, MIC_{90} values for *Enterobacterales* (0.5–1 µg/mL), *P. aeruginosa* (0.5 µg/mL) (0.25–0.5 µg/mL) did not witness any substantial change.[11–13] However, for *A. baumannii*, the MIC_{90} values increased from 1 µg/mL to 4µg/mL in each consecutive year of the SIDERO-WT study.[11–13] The reason for the high CFDC MIC of CR-GNB isolates in our study prior to drug exposure/usage needs additional investigation for the putative resistance mechanism.

In the present study, CFDC MIC_{50} and MIC_{90} values (4 and 128 µg/mL respectively) of CR-GNB isolates without five major carbapenemase genes were higher compared to MIC_{50} and MIC_{90} values (2 and 8µg/mL respectively) of CR-GNB isolates which harbored one or more carbapenemase gene. Among carbapenemase producers, bla_{NDM-1} harboring CR-GNB strains had two-fold higher MIC_{90} (16 µg/mL) than isolates bearing both NDM-1 and OXA48-like allele (8 µg/mL) or OXA48-like allele (8 µg/mL) alone. Additionally, MIC_{90} (>128µg/mL) of Non-CP-CRE was 8-fold higher than MIC_{90} (16µg/mL) of CP-CRE. Among NF-GNB, both MIC_{50} and MIC_{90} values (4 µg/mL,16µg/mL respectively) of isolates harboring no carbapenemase encoding gene were two folds higher than MIC_{50} and MIC_{90} values (2 µg/mL and 8 µg/mL respectively) of isolates harboring either one or more carbapenemase encoding genes. Morris et al also reported that the MIC_{90} value among non-carbapenemase-producing CRE (16 g/mL) was 4-fold higher than CP-CRE (4 g/mL).[7] In another study by Kazmierczak et al, 35.7% of the NDM-positive isolates were not inhibited by ≤4 mg/L of CFDC.[11] In SIDERO-WT and SIDERO-CR, MIC_{90} of bla_{NDM} harboring CRE (8 µg/mL) was twofold higher than MIC_{90} (4 µg/mL) of CRE encoding other carbapenemase genes (bla_{OXA48}, bla_{VIM}, bla_{KPC}).[11–13,20] In non-carbapenemase producing *E. coli* and *P. aeruginosa*, mutations in outer membrane iron transporters (eg CirA and Fiu in *E. coli* and PiuA in *P. aeruginosa*) were associated with high CFDC MICs (≥16-fold increase).[22,23] Additional mechanisms contributing to CFDC resistance in non-carbapenemase-producing GNB were not investigated in our study and thus cannot be commented upon.

Though a single-center study, this study is the first one to evaluate CFDC susceptibility in India, however, there are a few limitations. Only the five most common genes (bla_{NDM-1}, bla_{OXA-48}, bla_{KPC}, bla_{IMP}, bla_{VIM}) encoding for Carbapenem resistance were studied in 319 of the 503 CR-GNB. Other mechanisms leading to Carbapenem resistance, e.g., porin loss and efflux pump mechanisms were not studied. We used RUO disk of CFDC and CAMHB from single manufacturers.

Conclusion

We found that 81.7% of CR-GNB isolates had CFDC MIC in the susceptible range which was lower than published international studies, where susceptibility rates were >90%. Higher CFDC MIC_{50} and MIC_{90} values were found in non-carbapenemase-producing isolates compared to carbapenemase-producing CR-GNB isolates. DD using RUO disk had 9.1% VME, 1.45% ME, and 12.5% mE. Further, a large-scale study with a greater number of isolates, disks, and CA-MHB from different manufacturers is required to evaluate DD as an alternative method of BMD. The genetic mechanism of CFDC resistance needed to be performed to elucidate the mechanisms leading to high MIC in CR-GNB isolates.

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Disclosure

The authors report no conflicts of interest in this work.

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