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In vitro susceptibility of clinical *Clostridioides difficile* isolates in Israel to metronidazole, vancomycin, fidaxomicin, ridinilazole and ibezapolstat,

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Abstract

Background Antibiotics are currently the primary treatment of *Clostridioides difficile* (*C. difficile*) infection. Yet, due to rapid development of resistance and high recurrences rates, there is an unmet need for new antimicrobials for *C. difficile* infections. This study assessed the in vitro susceptibility of clinical isolates from Israel to two recently developed antibiotics, ridinilazole (RDZ) and ibezapolstat (IBZ), and to standard-of-care antibiotics.

Methods *C. difficile* isolates ($n=313$) recovered from patients at both community and hospital medical centers across Israel, were typed to different sequence types (ST) by multi-locus sequencing typing (MLST). Susceptibility to metronidazole (MTZ) and vancomycin (VAN) was determined using the gradient strip test (Etest). Susceptibility to fidaxomicin (FDX), RDZ and IBZ was determined by agar dilution.

Results ST42 (39; 12.5%) and ST2 (36; 11.5%) were the most prevalent STs. Resistance to MTZ and VAN was low (2.2%, 1.6%, respectively), while 23 (7.35%) isolates were FDX-resistant. RDZ MIC ranged between 0.06 and 0.5 mg/L, and $MIC_{50/90}$ was 0.25/0.5 mg/L. IBZ had an $MIC_{50/90}$ of 4 mg/L. No significant differences were noted in IBZ MIC of different strains.

Conclusions RDZ and IBZ demonstrated potent in vitro activity against 313 *C. difficile* isolates belonging to different STs. These two antimicrobials may serve as effective agents for *C. difficile* infection.

Keywords *C. difficile*, Antibiotic susceptibility, Fidaxomicin, Ridinilazole, Ibezapolstat, Strains, MLST

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Background

Clostridioides difficile (*C. difficile*) is a Gram-positive, anaerobic bacterium which causes significant diarrheal illness, both in healthcare and community facilities [1]. Since 2011, the Emerging Infections Program (EIP) of the Centers for Disease Control and Prevention (CDC) has been monitoring *C. difficile* infection (CDI) in 10 US sites. A recent analysis of EIP data found a 24% decrease in the total burden of CDI in the US between the years 2011 and 2017. Yet, there were no changes in the high burden of first recurrences and of CDI-associated in-hospital mortality [2].

CDI begins with ingestion of *C. difficile* spores, followed by their germination in the gut, which results in bacterial colonization and proliferation. The toxins produced by the bacteria disrupt the gut epithelial integrity, induce cytotoxic effects on intestinal cells and stimulate an inflammatory response [1]. The main risk factor for developing CDI is antibiotic use, as it induces dysbiosis, i.e., alteration of the gut microbiome composition, which enables germination of *C. difficile* spores [3]. Dysbiosis also drives an increased ratio of primary-to-secondary bile acids. As primary bile acids promote spore germination while secondary bile acids inhibit *C. difficile* growth, this shift contributes to CDI development [3].

Nonetheless, antibiotics are currently the primary treatment for CDI. The primary treatment options are fidaxomicin (FDX) and vancomycin (VAN); metronidazole (MTZ) may be given when the previous two antibiotics are not available [4]. While MTZ and VAN are effective against vegetative *C. difficile* cells, their use still induces gut microbiome disruption, which may lead to further *C. difficile* spore germination and to disease recurrence [5]. The advantages of FDX over VAN and MTZ are its longer duration of effect and reduced CDI recurrence rate [6]. Additionally, FDX use has been associated with less microbiome dysbiosis [6].

In recent years, several new *C. difficile* strains have emerged, some of which are resistant to antibiotics in clinical use. Additionally, up to 30% of treated patients may experience recurrent CDI due to persistence of antibiotic-resistant spores [7]. Thus, new alternatives are required. One of the newest antibiotics for CDI treatment is ridinilazole (RDZ), a narrow-spectrum bis-benzimidazole antibiotic. Its bactericidal activity is mediated by its interaction with AATTT-rich sequences in the *C. difficile* DNA minor groove, resulting in disruption of cell division and of ATP production [8].

In addition to its high inhibitory activity against several *C. difficile* strains, both in vitro and in vivo [9, 10], RDZ reduced CDI recurrence rate from 17.3% (with VAN) o 8.1%, ($p = 0.0002$) [8]. In contrast to VAN, RDZ does not impact the gut microbiome [11, 12] and has no effect on secondary bile acids [12]. Two clinical trials (NCT03595553 and CT03595566) comparing the efficacy of RDZ vs. VAN, found better conservation of gut microbiome with RDZ [8]. RDZ treatment in a phase 3 superiority trial was associated with less recurrence cases and increased secondary bile acids levels [8]. Yet, a phase 3 study of RDZ was recently terminated due to failure to show superiority of RDZ to VAN in sustained clinical response (73%, and 70.7%, respectively); the drug company which developed RDZ stated on rethinking [8].

Ibezapolstat (IBZ) is another treatment recently developed for CDI. This narrow-spectrum antibiotic binds and inhibits DNA polymerase IIIC (DNA pol IIIC), which is unique to Gram-positive bacteria with a low G + C content. IBZ has exhibited several advantages, including minimal adverse effects, good pharmacokinetics, a favourable secondary-to-primary bile acid ratio and limited damage to the gut microbiome [13]. Murray et al. reported on the potent activity of IBZ against 104 *C. difficile* isolates [14]. Recently, a phase 2b clinical trial for assesment of the clinical efficacy of IBZ as a treatment of CDI patients has been completed. Clinical cure at Day 12 was achieved for 15 out of 18 (83.3%) patients treated with IBZ. Additionaly, no recurrences were observed for 93.8% of patients for 38 days [15].

To the best of our knowledge, there are no data regarding susceptibility of clinical *C. difficile* strains in Israel to RDZ and IBZ. Additionally, since antibiotic susceptibility of *C. difficile* is not routinely tested, there are limited data on susceptibility rates to the currently used treatments. This study assessed the susceptibility of different clinical strains collected from several areas in Israel between 2020 and 2022, to RDZ, IBZ, FDX, MTZ and VAN.

Methods

Study isolates

C. difficile isolates were recovered from stool samples of patients diagnosed with CDI and hospitalized in one of four medical centres in Israel between 2020 and 2022. CDI was confirmed with the GeneXpert *C. difficile* BT PCR assay (Cepheid, Sunnyvale, CA, USA), which identifies toxin B and binary toxin genes, as well as *tcd*C deletion (for identification of the epidemic Nap1/027 strain). Community-acquired CDI (CA-CDI) was defined as CDI that developed within 48 h of admission, while hospital-acquired CDI (HA-CDI) was defined as CDI that developed > 48 h after admission [16].

The four participating medical centres are located in different geographic areas of Israel: North - Tzafon Medical Center, Poriya and W. Hirsch Regional Microbiology Laboratory Clalit Health Services, Haifa, Center - Edith Wolfson Medical Center, Holon, and South - Soroka University Medical Center, Be'er Sheva. The local Ethics (Helsinki) Committee of each medical centre approved

the study (POR-0085-15, WOMC-0115-20, SOR-0307-20). The need for informed consent was waived.

Bacterial isolation and identification

Stool samples were inoculated on chromID™ *C. difficile* (CDIF) (BioMérieux, Durham, NC), and incubated for 48 h, at 37 °C, in a Bactron EZ 300 anaerobic chamber (Sheldon Manufacturing, Cornelius, USA). Identification of *C. difficile* colonies was based on their typical asymmetrical shape and black color. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry analyses were performed using a Bruker Biotyper system (Bruker Daltonics, Bremen, Germany), for definitive identification. All isolates were stored at -80 °C until further analysis.

Multi-locus sequence typing (MLST)

DNA was extracted from study isolates using the MagCore® Genomic DNA Bacterial Kit (ATRIDAB.V, Amersfoort, Netherlands), with the MagCore® automated extraction instrument (RBCBioscience, New Taipei, Taiwan), according to the manufacturer's instructions. Following whole-genome sequencing of DNA samples, the sequences of seven housekeeping genes (*adk*, *atp*A, *dxr*, *gly*A, *rec*A, *sod*A, and *tpi*) of each isolate were uploaded to the *C. difficile* MLST database (https://pubmlst.org/organisms/clostridioides-difficile), in order to determine the sequence type (ST), as previously described [17].

Antimicrobial susceptibility testing

MTZ and VAN susceptibility testing

Susceptibility to MTZ and VAN was assessed using a gradient strip, which determines the minimum inhibitory concentration (MIC) of each antibiotic. Several *C. difficile* colonies were suspended in thioglycollate broth medium (Becton Dickinson, Heidelberg, Germany) to achieve 1.0 McFarland standards. Then, bacterial suspensions were inoculated on Brucella blood agar supplemented with hemin and vitamin K1 (Hy Laboratories, Rehovot, Israel) and a VAN or MTZ gradient Etest strip (bioMérieux, Durham, NC) was added. The agar plates were incubated at 37 °C, under anaerobic conditions, for 48 h. Following incubation, the MIC was visually determined and isolates were classified as susceptible or resistant according to ECOFFs of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [18]. *C. difficile* ATCC 700,057 was used for a quality control.

Table 1 Distribution of ST among study isolates

ST	Clade	*n* (%)
ST42	1	39 (12.5)
ST2	1	36 (11.5)
ST104	1	23 (7.3)
ST11	5	21 (6.7)
ST3	1	13 (4.2)
ST34	1	9 (2.9)
ST54	1	9 (2.9)
ST55	1	9 (2.9)
ST37	4	8 (2.6)
ST13	1	7 (2.2)
Others	1, 2, 3, 4	108 (34.5)
Unclassified	N.A.	31 (9.9)

FDX, RDZ and IBZ susceptibility testing

Susceptibility to FDX, RDZ and IBZ was assessed in accordance with the procedures of the Clinical and Laboratory Standards Institute (CLSI-M11-9th) [19]. Brucella agar supplemented with 5% defibrinated sheep blood (Hy Laboratories), was mixed with FDX (Sigma-Aldrich, Missouri, US), RDZ or IBZ (MedChemExpress LLC, NJ, USA) by first dissolving the antibiotic in dimethyl sulfoxide (DMSO) and then further diluting it with distilled water to the desired concentrations. The agar plates were mixed with the different dilutions of each antibiotic, yielding the following ranges of final concentrations: FDX 0.03-32 mg/L, RDZ-0.03-0.5 mg/L, and IBZ- 0.5-8 mg/L.

Several *C. difficile* colonies were inoculated in thioglycollate broth medium (Becton Dickinson) to 0.5 McFarland turbidity, and then placed as spots on the antibiotics-supplemented agar plates. Plates were incubated at 35 °C, under anaerobic conditions, for 48 h. After incubation, plates were visually screened for bacterial growth, and MIC was determined as the lowest antibiotic concentration that inhibited 90% of bacterial growth.

Results

The study included 313 isolates, recovered from 187 patients with HA-CDI and 126 patients with CA-CDI.

ST distribution

ST was determined for 90.1% (282/313) of the isolates. Isolates were categorized into ten major groups, with each group containing at least seven isolates (Table 1). An additional group, called "others", included 108 (34.5%) isolates with STs shared by fewer than seven isolates (Supplemental Table 1).

The most prevalent STs were ST42 ($n=39$; 12.5%) and ST2 ($n=36$; 11.5%). Most isolates (274/282; 97.2%) belonged to Clade 1. One ST1 isolate belonged to Clade 2, two ST5 isolates belonged to Clade 3, nine isolates (8 ST37 and 1 ST39) belonged to Clade 4 and 21 ST11isolates belonged to Clade 5. The "Others" group included isolates from Clades 1–4 (Table 1). One hypervirulent strain (0.3%), belonging to ST1, was found.

Overall, the same STs were found among both CA and HA isolates, however, their distributions differed (Fig. 1). For example, among HA isolates, ST42 was the most common strain (13.9%), while ST2 was the most

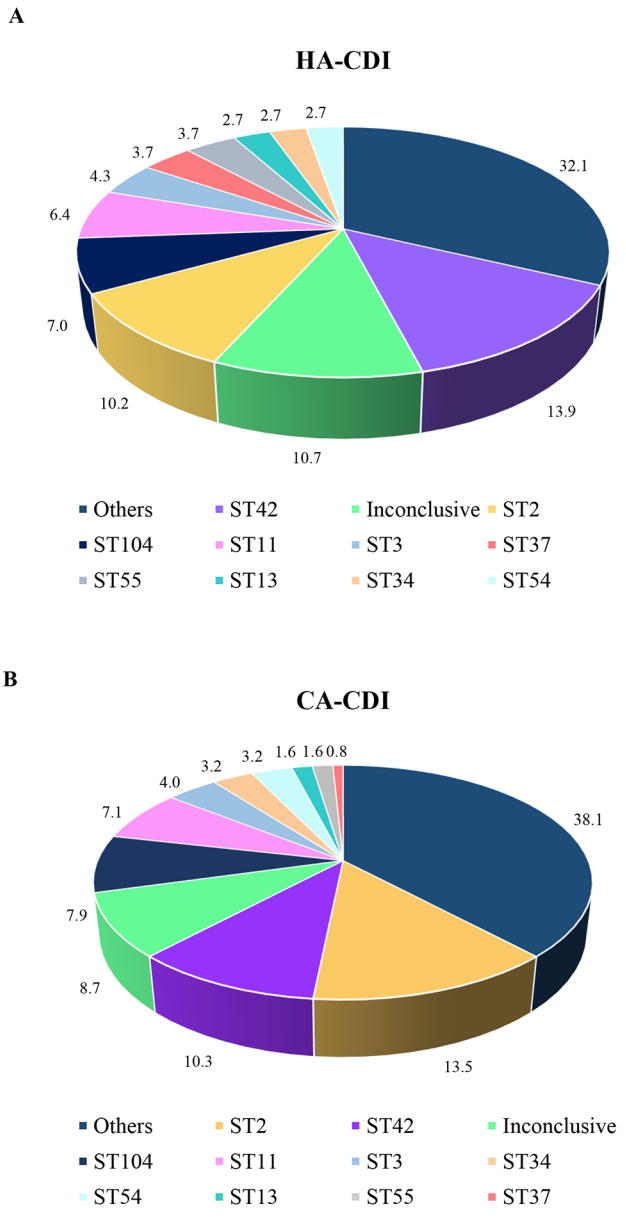

Fig. 1 Distribution of *C. difficile* sequence types (STs) among study isolates

frequent ST (13.5%) among CA isolates. ST37 was more frequently detected among HA isolates compared to CA isolates (3.7%, 0.8%, respectively).

C. difficile isolates were typed by MLST. The figure presents the distribution of STs among *C. difficile* isolates that were recovered from stool samples of patients with (a) HA-CDI ($n = 187$), (b) CA-CDI ($n = 126$).

Susceptibility of study isolates

Bacterial susceptibility to MTZ, VAN and FDX, and to the new antimicrobials RDX and IBZ was assessed (Table 2; Fig. 2). The MICs of MTZ were in the range of 0.016-256 mg/L and $MIC_{50/90}$ was 0.19/0.38 mg/L. The resistance rate to MTZ was low (2.2%). The MIC_{50} of VAN was 0.5 mg/L, the MIC_{90} was 0.75 mg/L, and the resistance rate was low (1.6%). The geometric MIC mean of both MTZ and VAN was quite high (6 mg/L and 2.5 mg/L, respectively). FDX MIC was in the range of 0.03-16 mg/L and $MIC_{50/90}$ was 0.25/0.5 mg/L. Twenty three (7.35%) isolates were resistant to FDX. RDX MIC ranged between 0.06 mg/L and 0.5 mg/L, and the $MIC_{50/90}$ was 0.25/0.5 mg/L. IBZ had an $MIC_{50/90}$ of 4 mg/L.

The figure presents the distribution of MIC values of the following antibiotics (a) metronidazole, (b) vancomycin, (c) fidaxomicin, (d) ridinilazol and (e) ibezapolstat in study isolates. Susceptibility to antibiotics was determined by the E test or by agar dilution methods. The dashed line represents the breakpoints for resistance determination (MIC > 2 mg/l for MTZ and VAN, and MIC > 0.5 mg/L for FDX).

No major differences in MIC of the antibiotics, including RDX and IBZ (Table 3) were noted across the different STs. Furthermore, no differences were observed between the $MIC_{50}/_{90}$ and geometric mean MIC for RDZ and IBZ of MTZ-susceptible and MTZ-resistant strains or of VAN-susceptible and VAN-resistant strains (Table 4). In addition, when comparing the current findings with data from all studies that tested the in vitro activity of RDZ and/or IBZ (Table 5), the RDZ MICs in the current analysis were among the highest reported values. In most studies, RDZ had a lower $MIC_{90,}$ compared to MTZ and VAN. Furthermore, RDZ had either lower or equal MIC_{90} as IBZ. IBZ $MIC_{50}/_{90}$ in the current study were similar to those reported in a recent study conducted in the USA

Table 2 Antimicrobial susceptibility of study isolates

Antimicrobial agent	MIC Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Geometric MIC mean (mg/L)	% Resistance
Metronidazole	0.016-256	0.19	0.38	6	2.2
Vancomycin	0.064-256	0.5	0.75	2.5	1.9
Fidaxomicin	0.03-16	0.25	0.5	0.81	7.35
Ridinilazole	0.06–0.5	0.25	0.5	0.32	N.A.
Ibezapolstat	0.5-8	4	4	3.1	N.A.

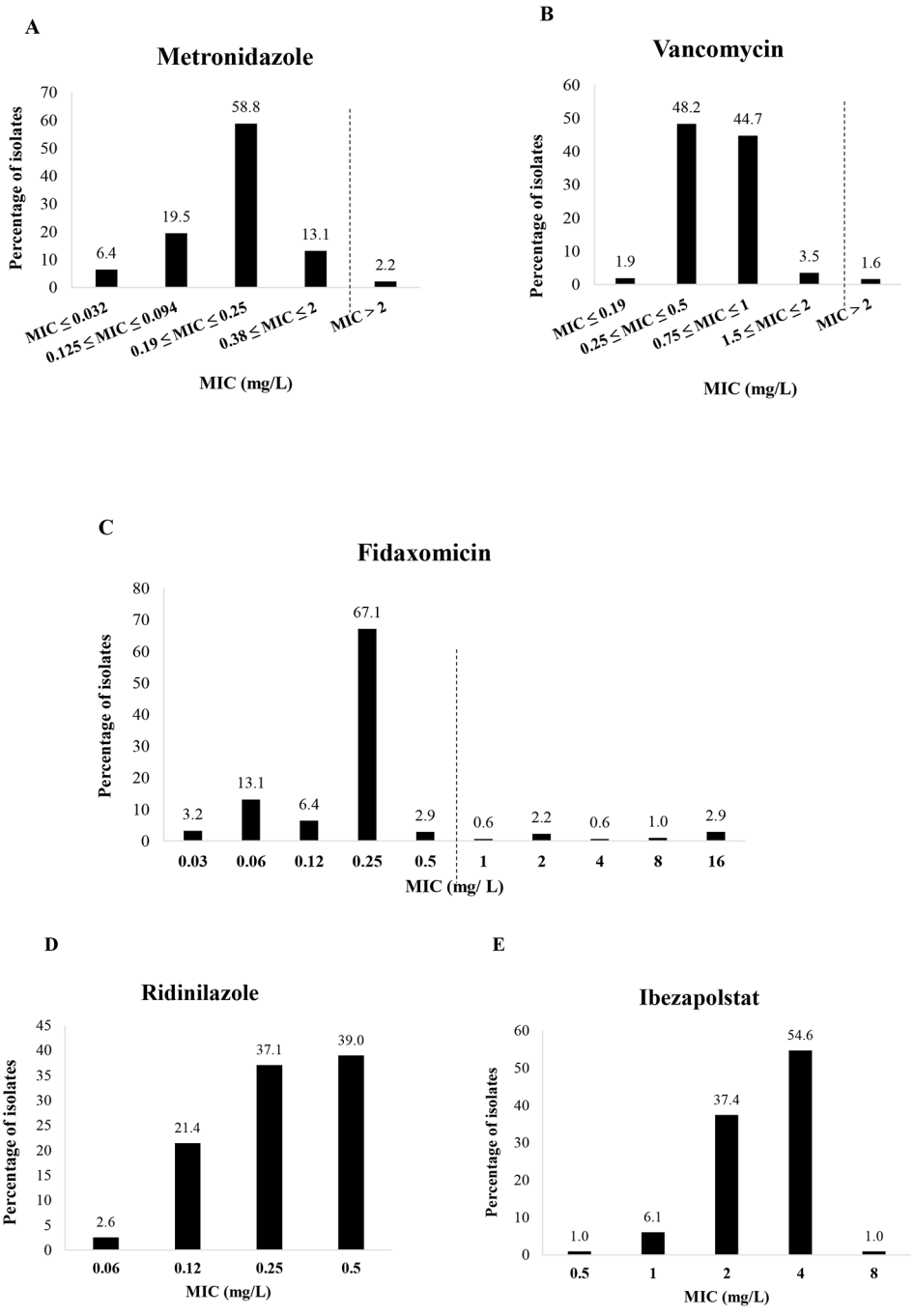

Fig. 2 Distribution of MIC of different antibiotics in *C. difficile* isolates

(Table 5). Out of the four studies evaluating IBZ, only two, including the current study, tested other antibiotics as well. In these two studies, IBZ MIC_{90} was higher than the VAN, MTZ and FDX MIC_{90}.

Discussion

The current study investigated the susceptibility of 313 *C. difficile* isolates collected from patients across Israel, to the recently developed antimicrobials RDZ and IBZ, as well as to standard-of-care antibiotics.

ST distribution

ST42 and ST2 were the predominant STs in the current study. ST42 which corresponds with Ribotype106/

Table 3 Antibiotic susceptibility of study isolates, with relation to ST

Strains	MIC (μg/mL)	MTZ	VAN	FDX	RDZ	IBZ
ST2	Range	0.032-1	0.094-2	0.03-16	0.12–0.5	2–4
(*n* = *36)*	MIC_{50}	0.125	0.5	0.25	0.25	4
	MIC_{90}	0.25	0.75	0.25	0.5	4
ST3	Range	0.023-0.2	0.25–0.75	0.03-16	0.12–0.25	1–4
(*n* = *13*	MIC_{50}	0.094	0.75	0.12	0.25	4
	MIC_{90}	0.25	0.75	0.25	0.5	4
ST11	Range	0.016-256	0.064-256	0.03-16	0.12–0.5	2–4
(*n* = *21)*	MIC_{50}	0.094	0.75	0.12	0.5	2
	MIC_{90}	0.25	1	0.25	0.5	4
ST13	Range	0.125–0.25	0.5–0.75	0.06-16	0.12–0.5	2–4
(*n* = *7)*	MIC_{50}	0.19	0.5	0.25	0.5	4
	MIC_{90}	0.25	0.75	0.25	0.5	4
ST34	Range	0.032–0.38	0.5-3	0.06-8	0.06–0.5	2–4
(*n* = *9)*	MIC_{50}	0.19	0.75	0.25	0.25	4
	MIC_{90}	0.38	0.75	0.25	0.5	4
ST37	Range	0.016-256	0.5–0.75	0.06–0.25	0.06–0.5	1–4
(*n* = *8)*	MIC_{50}	0.19	0.75	0.25	0.25	4
	MIC_{90}	0.75	0.75	0.25	0.5	4
ST42	Range	0.016-256	0.25-56	0.03-4	0.06–0.5	0.5-4
(*n* = *39)*	MIC_{50}	0.25	0.75	0.25	0.25	4
	MIC_{90}	0.75	0.75	0.25	0.5	4
ST54	Range	0.023–0.38	0.38-1	0.06–0.25	0.12–0.5	1–4
(*n* = *9)*	MIC_{50}	0.19	0.75	0.25	0.25	2
	MIC_{90}	0.25	1	0.25	0.5	4
ST55	Range	0.064–0.38	0.5–0.75	0.12–0.25	0.25–0.5	4–8
(*n* = *9)*	MIC_{50}	0.125	0.75	0.25	0.25	4
	MIC_{90}	0.25	0.75	0.25	0.5	4
ST104	Range	0.023-1	0.38-1	0.03-16	0.06–0.5	1–4
(*n* = *23)*	MIC_{50}	0.19	0.5	0.25	0.25	2
	MIC_{90}	0.25	1	0.5	0.5	4
Unclassified	Range	0.047-256	0.125-2	0.06-16	0.06–0.5	1–4
(*n* = *31)*	MIC_{50}	0.19	0.5	0.25	0.5	2
	MIC_{90}	0.25	0.75	8	0.5	4
Others	Range	0.016-256	0.125-256	0.03-16	0.06–0.5	0.5-8
(*n* = *108)*	MIC_{50}	0.19	0.75	0.25	0.25	2
	MIC_{90}	0.38	0.75	0.25	0.5	4

Table 4 Susceptibility of study isolates to RDZ and IBZ, with relation to their susceptibility to MTZ, VAN and FDX

Antimicrobial agent	RDZ			IBZ		
	MIC_{50}	MIC_{90}	Geometric MIC mean	MIC_{50}	MIC_{90}	Geometric MIC mean
(mg/L)						
Metronidazole-S	0.5	0.5	0.313	4	4	3.07
Metronidazole-R	0.5	0.5	0.41	4	4	3
Vancomycin-S	0.25	0.5	0.314	4	4	3.07
Vancomycin-R	0.5	0.5	0.374	4	4	3.2
Fidaxomicin-S	0.25	0.5	0.31	4	4	3.06
Fidaxomicin-R	0.5	0.5	0.37	4	4	3.21

RT106, has been reported worldwide. For example, in the US, RT106 was the second most detected strain in 2012 and the most prevalent strain in 2016 [20, 21]. Most RT106 isolates are susceptible to MTZ and VAN [21].

Regarding ST2, a study performed in a Chinese hospital found ST2 to be the second-most-prevalent ST (10, 11.11%) among the 90 isolated strains analyzed [22]. Both RT106/ST42 and ST2 are toxins A and B producers [22].

Interestingly, a previous study conducted by our group, which characterized 70 *C. difficile* isolates collected during the years 2016–2018, found ST4 (22.5%) and ST37 (12.7%) to be the most common STs [23]. Although the study was performed in a single geographic area in Israel,

Table 5 Summary of reported data regarding *C. difficile* susceptibility to MTZ, VAN, FDX, RDZ and IBZ

Reference (Geographic area)	No. of isolates	MIC (µg/mL)	IBZ	RDZ	FDX	VAN	MTZ
Dvoskin et al., 2012 (USA) [36]	23	Range	Not shown	N.D.	N.D.	N.D.	N.D.
		MIC_{50}	2				
		MIC_{90}	4				
Goldstein et al., 2013 (USA) [34]	50	Range	N.D.	0.125-0.5	0.06-1	1–8	0.25-8
		MIC_{50}		0.25	0.25	1	0.5
		MIC_{90}		0.25	0.5	4	2
Corbett at al., 2015 (UK) [37]	82	Range	N.D.	0.06–0.125	0.008–0.125	0.5-4	0.125-8
		MIC_{50}		0.125	0.03	1	2
		MIC_{90}		0.125	0.06	2	8
Freeman et al., 2016 (Europe) [38]	107	Range	N.D.	0.015-0.5	0.004–0.125	0.5-8	< 0.125-2
		MIC_{50}		0.03	0.06	1	0.2
		MIC_{90}		0.125	0.125	2	2
Snydman et al., 2017 (US) [39]	200	Range	N.D.	0.12–0.5	0.015-1	0.25-4	0.12-2
		MIC_{50}		0.12	0.03	1	0.25
		MIC_{90}		0.25	0.125	2	1
Snydman et al., 2018 (US) [11]	44	*Range	N.D.	0.06–0.5	0.06-1	1–4	0.12-4
		MIC_{50}		0.12	0.12	1	0.5
		MIC_{90}		0.25	0.5	2	2
	45	#Range		0.06–0.5	0.06-1	0.5-2	0.12-2
		MIC_{50}		0.12	0.25	1	0.25
		MIC_{90}		0.5	0.5	2	1
van Eijk, et al., 2019 (the Netherlands) [33]	363	Range	0.5-4	N.D.	N.D.	N.D.	N.D.
		MIC_{50}	2				
		MIC_{90}	4				
Murray et al., 2020 (USA) [14]	104	Range	1–8	N.D.	0.015-1	0.5-4	0.25-16
		MIC_{50}	4		0.12	1	0.5
		MIC_{90}	4		0.25	2	1
Collins et al., 2021 (Japan, China, South Korea) [30]	140	Range	N.D.	0.03–0.25	0.015–0.25	0.06-4	0.06–0.5
		MIC_{50}		0.125	0.125	1	0.25
		MIC_{90}		0.25	0.25	2	0.25
Snydman et al., 2023 (US) [29]	300	Range	N.D.	0.3–0.5	0.03–0.5	0.25-4	0.12-4
		MIC_{50}		0.25	0.25	2	0.5
		MIC_{90}		0.25	0.5	2	1
Bassères et al., 2024 (US) [32]	100	Range	-	N.D.	-	-	-
		MIC_{50}	4		0.5	2	0.25
		MIC_{90}	8		1	4	4
The current study (Israel)	313	Range	0.5-8	0.06–0.5	0.03-16	0.064-256	0.016-256
		MIC_{50}	4	0.25	0.25	0.5	0.19
		MIC_{90}	4	0.5	0.5	0.75	0.38

N.D., not detected

the different distribution of STs as compared to the current study suggest that CDI epidemiology is changing in Israel. Of note, studies from other Middle East countries reported different strain distribution (for review, see Brajerova et al., 2022 [24].

Among the current subset of HA isolates, ST42 was more common than ST2. The opposite order was seen among CA-CDI isolates, where ST2 was the most detected strain, followed by ST42. Several studies suggested that RT014/RT020/ST2 has a community origin [25, 26], a hypothesis that was strengthened by the current results. Furthermore, RT014/ST2 isolates have been recovered from various environmental sources, including wastewater [27], parks and homes [28], further strengthening evidence for a community origin for this strain.

Susceptibility of study isolates to antibiotics in clinical use

Resistance rates to the tested isolates to MTZ and VAN were low (2.2% and 1.6%, respectively). A previous study by our group, which characterized 70 isolates from north Israel, reported on considerably higher (17.1%) resistance rate to MTZ and a similar resistance rate (1.4%) to VAN [23]. As suggested above, these differences suggest an evolving epidemiology of *C. difficile* in Israel. Lower resistance rates to both MTZ (0.3%) and VAN (0.7%) were reported in a recent US study of 300 *C. difficile* isolates [29]. Collins et al., who investigated the

susceptibility of 140 *C. difficile* isolates from Japan, China and South Korea, to various antibiotics, did not find any isolates resistant to either MTZ or VAN [30]. The low resistance rates measured in the current study may be the result of decreased use of MTZ and VAN in recent years, due to the introduction of FDX into clinical use.

FDX MIC_{50} in the current study was low (0.25 µg/mL) but FDX MICs were higher (the maximum MIC was 32 µg/mL) than those reported in recent studies (see Table 5). Although there is no clinical breakpoint for FDX, the 0.5 mg/L ECOFF proposed by EUCAST suggests that there are already FDX-resistant strains in Israel. Yet, according to a recent systematic review and meta-analysis of 1184 isolates, the resistance rate to FDX based on a breakpoint of ≥ 8 mg/L, was 0.08% [31]. FDX susceptibility should be further monitored for early recognition of resistant strains and treatment failure.

Susceptibility of study isolates to RDZ and IBZ

Overall, low RDZ and IBZ MICs were measured. The two antibiotics were effective against all STs and all isolates with different susceptibilities to MTZ/VAN/FDX. Findings relating to IBZ strengthen previous reports that showed that IBZ was equally effective across different ribotypes and strains with different MTZ/VAN/FDX susceptibility patterns [32, 33]. When comparing the present results with recent studies that investigated RDZ susceptibility, the MICs in the current study were among the highest reported values. For example, most studies reported on MIC_{90} of ≤ 0.25 mg/L, while in the current study, the MIC_{90} was 0.5 mg/L. Currently, there are no breakpoints for this new antimicrobial; further studies should be performed to gain sufficient and a comprehensive data regarding *C. difficile* susceptibility to RDZ.

Comparison of RDZ activity to that of other antibiotics found that the RDX MIC_{90} was generally lower or similar to the MIC_{90} of FDX and always lower than those of VAN and MTZ. Furthermore, RDZ was less effective against Gram-negative anaerobes and Gram-positive aerobes, as compared to FDX, VAN and MTZ [34]. Thus, the superiority of RDZ over the currently used antibiotics manifests not only by its increased potency against *C. difficile*, but also by its reduced effect on the gut microbiome. It should be noted that the RDZ manufacturer terminated clinical trials due to non-superiority of RDZ to VAN with regards to sustained clinical response and is considering modifying the molecule [35].

The IBZ MIC_{50} and MIC_{90} for isolates in the current study aligned with those previously reported by others. However, only a small number of studies reported on the susceptibility of *C. difficile* to this antimicrobial in vitro. Thus, additional studies are still needed to compare the susceptibility of isolates from different geographic areas.

Of note, IBZ had a wider MIC range and higher $MIC_{50/90}$ values as compared to RDZ, which may indicate increased potency of RDZ as compared to IBZ. However, as this study was the first to test both RDZ and IBZ activity, further investigations will be necessary to confirm this suggested improved effectiveness. IBZ MICs were also higher than those of FDX, MTZ and VAN. However, the reduced adverse effects and limited interruption to gut microbiome [13] outweigh the high dose requirement.

Conclusions

This study demonstrated the potent in vitro activity of RDZ and IBZ against 313 *C. difficile* isolates belonging to different STs and clades. To date, the two antimicrobials has proven ideal for CDI treatment, with excellent activity against *C. difficile* and minimal impact on bacterial species that comprise the gut microbiome.

Abbreviations

CA	community-acquired
CDI	C. difficile infection
FDX	fidaxomicin
HA	hospital-acquired
IBZ	ibezapolstat
MALDI-TOF	matrix-assisted laser desorption ionization-time of flight
MIC	minimum inhibitory concentration
MLST	multi-locus sequencing typing
MTZ	metronidazole
RDZ	ridinilazole
ST	sequence type
VAN	vancomycin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-025-03800-7.

Supplementary Material 1

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Not applicable.

Author contributions

O.S. collected the data, performed the experiments, analyzed results and wrote the manuscript. M.A. and A.P. were major contributors in result analysis and the manuscript writing. All authors read and approved the final manuscript.

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Data availability

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request. DNA sequences were deposited in NCBI repository, accession number PRJNA1044122 (accession numbers of isolates SRR26919831- SRR26920158).

Declarations

Ethics approval and consent to participate

The study adhered to the Declaration of Helsinki. Tzafon Medical Center, Poriya and W. Hirsch Regional Microbiology Laboratory Clalit Health Services, Haifa, Center - Edith Wolfson Medical Center, Holon, and South - Soroka University Medical Center, Be'er Sheva. The local Ethics (Helsinki) Committee of Tzafon Medical Center, W. Hirsch Regional Microbiology Laboratory Clalit Health Services and Soroka University Medical Center approved the study (approval numbers-POR-0085-15, WOMC-0115-20, SOR-0307-20, respectively). The need for informed consent was waived by all three mentioned committees. All experiments were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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