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Characterization of *Clostridioides difficile* Isolates Available through the CDC & FDA Antibiotic Resistance Isolate Bank

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ABSTRACT Thirty *Clostridioides difficile* isolates collected in 2016 through the Centers for Disease Control and Prevention Emerging Infections Program were selected for reference antimicrobial susceptibility testing and whole-genome sequencing. Here, we present the genetic characteristics of these isolates and announce their availability in the CDC & FDA Antibiotic Resistance Isolate Bank.

Clostridioides difficile infection (CDI) is among the most common health care-associated infections in the United States, linked to approximately 30,000 deaths annually (1). Antibiotic exposure is a risk factor for CDI, and resistance to commonly used antimicrobials may contribute to the evolving epidemiology of *C. difficile* (1). The Centers for Disease Control and Prevention (CDC) performs surveillance through the Emerging Infections Program (EIP) to monitor CDI in the United States (https://www.cdc.gov/hai/eip/cdiff-tracking.html). We announce a panel of 30 community- and health care-associated EIP CDI isolates, representing the 10 most prevalent PCR ribotypes (RTs) collected in 2016, available through the CDC & FDA Antibiotic Resistance Isolate Bank (AR Isolate Bank, https://wwwn.cdc.gov/ARIsolateBank/).

Strain typing was performed using a standardized high-resolution capillary gel-based PCR ribotyping protocol (2) with an in-house curated standard profile library and 100% similarity threshold (BioNumerics 6.6.11; Applied Maths). Multiplex real-time PCR (3) was used to detect genes encoding cytotoxins A and B (*tcdA*, *tcdB*) and binary toxin (*cdtA*, *cdtB*). Deletions in anti-sigma factor *tcdC* were assessed by PCR fragment (3) and whole-genome sequencing (WGS) analysis. For WGS, genomic DNA was extracted from colonies cultured overnight on CDC anaerobic blood agar with vitamin K, hemin, and 5% sheep blood at 37°C under anaerobic conditions from 10% skim milk glycerol stocks. DNA was isolated using the Promega Maxwell 16-cell low elution volume DNA purification kit and the Maxwell 16 MDx instrument (Madison, WI). DNA was sheared using the Covaris ME220 focused ultrasonicator (Woburn, MA), and indexed libraries were prepared using the NuGEN Ovation ultralow system version 2 assay kit (San Carlos, CA) and the PerkinElmer Zephyr G3 next-generation sequencing (NGS) workstation (Waltham, MA). Libraries were analyzed using the standard-sensitivity NGS fragment analysis kit and fragment analyzer system (Agilent Technologies, Santa Clara, CA). Sequencing was performed using the MiSeq reagent kit version 2 (500 cycles) and MiSeq system (Illumina, San Diego, CA), generating 2 × 250-bp paired-end reads. Sequence data were analyzed using the QuAISAR-H pipeline (https://github.com/DHQP/QuAISAR_singularity, version 1.0.2, default settings, accessed May 2019), including removal of phiX reads using BBDuk (BBMap version 37.87, sourceforge.net/projects/bbmap/), removal of adaptors and read trimming using Trimmomatic version 0.36 (4), and *de novo* assembly using SPAdes version 3.13.0 (5). Sequence types were

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TABLE 1 Molecular characteristics and antimicrobial susceptibility profiles for *C. difficile* isolates in the AR Isolate Bank[a]

Isolate no.	BioSample no.	RT	MLST	Toxin profile	*tcdC* mutation(s)[b]	MIC (μg/ml) for:						Mutation and/or gene(s) identified by ResFinder and ARG-ANNOT[c]	No. of reads	Genome size (bp)	No. of contigs	N_{50} (bp)	GC content (%)
						CRO	CLI	MEM	MTZ	MXF	VAN						
AR-1067	SAMN13029148	027	ST1	*tcdA/B*$^+$, *cdtA/B*$^+$	18bp, Δ117	64	>16	2	1	>8	2	*cfrC*, *dfrf*, *ermB*, *tetM*, *vanG*, *vanR*, *vanS*, *vanT*, *vanZ*, *gyrA* (Thr82Ile)	1,232,466	4,243,914	301	24,052	28.7
AR-1068	SAMN13029149	056	ST34	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	64	4	1	0.25	1	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,520,666	4,140,240	323	27,508	28.4
AR-1069	SAMN13029150	015	ST10	*tcdA/B*$^+$, *cdtA/B*$^-$	18bp	32	2	2	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,155,646	4,340,988	57	227,612	28.2
AR-1070	SAMN13029151	002	ST8	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	4	1	0.25	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,484,766	4,272,556	231	41,015	28.4
AR-1071	SAMN13029152	027	ST1	*tcdA/B*$^+$, *cdtA/B*$^+$	18bp, Δ117	64	8	2	1	8	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*, *gyrA* (Thr82Ile)	1,409,698	4,181,997	267	34,074	28.6
AR-1072	SAMN13029153	027	ST1	*tcdA/B*$^+$, *cdtA/B*$^+$	18bp, Δ117	64	>16	2	1	>8	2	*cfrC*, *ermB*, *vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*, *gyrA* (Thr82Ile)	1,826,800	4,172,909	222	38,400	28.6
AR-1073	SAMN13029154	020	ST2	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	4	2	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,579,584	4,177,999	117	103,880	28.6
AR-1074	SAMN13029155	002	ST8	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	8	2	0.5	8	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*, *gyrA* (Thr82Ile)	1,599,852	4,125,816	269	37,978	28.2
AR-1075	SAMN13029156	019	ST67	*tcdA/B*$^+$, *cdtA/B*$^+$	WT	32	4	2	0.5	1	0.5	*vanZ1*	1,604,652	4,207,539	53	163,554	28.5
AR-1076	SAMN13029157	027	ST1	*tcdA/B*$^+$, *cdtA/B*$^+$	18bp, Δ117	64	8	4	0.25	>8	2	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*, *gyrA* (Thr82Ile)	1,871,410	4,107,095	47	174,380	28.5
AR-1077	SAMN13029158	078	ST11	*tcdA/B*$^+$, *cdtA/B*$^+$	39bp, C184T	32	4	1	0.25	1	0.5	*ant(6)-ia*, *spw*, *tetM*	1,451,232	3,918,602	121	64,610	28.5
AR-1078	SAMN13029159	106	ST42	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	8	2	0.5	2	1	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,432,018	4,046,102	97	78,397	28.5
AR-1079	SAMN13029160	056	ST34	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	2	2	0.25	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,540,522	4,177,894	144	70,736	28.5
AR-1080	SAMN13029161	020	ST2	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	16	8	1	0.25	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*	1,326,024	4,145,293	256	28,424	28.7
AR-1081	SAMN13029162	014	ST110	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	2	1	0.25	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,596,282	4,091,127	79	125,940	28.6
AR-1082	SAMN13029163	054	ST43	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	1	2	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*	1,606,932	4,187,731	92	100,411	28.3
AR-1083	SAMN13029164	078	ST11	*tcdA/B*$^+$, *cdtA/B*$^+$	39bp, C184T	32	4	1	0.25	1	0.5	*ant(6)-ia*, *ant(6)-ib*, *spw*, *tetM*, *tet44*	1,076,082	3,964,561	153	56,789	28.5
AR-1084	SAMN13029165	002	ST8	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	2	1	0.25	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,887,930	4,039,601	47	171,828	28.2
AR-1085	SAMN13029166	106	ST42	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	64	8	2	0.25	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,514,658	4,119,511	223	42,869	28.5
AR-1086	SAMN13029167	015	ST10	*tcdA/B*$^+$, *cdtA/B*$^-$	18bp	32	4	1	0.25	1	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,520,910	4,319,533	122	73,649	28.3
AR-1087	SAMN13029168	106	ST42	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	2	1	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,670,654	4,022,519	63	151,217	28.4
AR-1088	SAMN13029169	054	ST43	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	8	2	0.25	1	0.5	*vanG*, *vanR*, *vanS*, *vanT*	1,178,682	4,094,056	100	78,787	28.3
AR-1089	SAMN13029170	106	ST42	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	64	>16	2	0.5	2	0.5	*ermB*, *vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	2,200,272	4,081,462	51	154,757	28.5
AR-1090	SAMN13029171	014	ST14	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	32	>16	2	0.5	2	0.5	*ermB*, *vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	2,200,528	4,155,416	68	119,271	28.6
AR-1091	SAMN13029172	014	ST2	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	64	4	2	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*	2,044,134	4,141,305	59	150,646	28.6
AR-1092	SAMN13029173	027	ST1	*tcdA/B*$^+$, *cdtA/B*$^+$	18bp, Δ117	64	>16	4	1	>8	1	*ermB*, *vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*, *gyrA* (Thr82Ile)	1,720,350	4,134,672	64	122,158	28.6
AR-1093	SAMN13029174	106	ST42	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	64	4	2	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*	1,201,366	4,071,812	146	62,561	28.5
AR-1094	SAMN13029175	019	ST67	*tcdA/B*$^+$, *cdtA/B*$^+$	WT	32	4	1	0.25	1	0.5	*vanZ1*	1,935,084	4,139,106	123	74,476	28.4
AR-1095	SAMN13029176	027	ST1	*tcdA/B*$^+$, *cdtA/B*$^+$	18bp, Δ117	128	4	2	0.5	>8	2	*vanG*, *vanR*, *vanS*, *vanT*, *vanZ1*, *gyrA* (Thr82Ile)	925,204	4,103,471	109	91,825	28.5
AR-1096	SAMN13029177	020	ST2	*tcdA/B*$^+$, *cdtA/B*$^-$	WT	64	8	2	0.5	2	0.5	*vanG*, *vanR*, *vanS*, *vanT*	1,946,384	4,095,571	38	204,538	28.6

[a] Abbreviations: CRO, ceftriaxone; CLI, clindamycin; MEM, meropenem; MTZ, metronidazole; MXF, moxifloxacin; VAN, vancomycin; MLST, multilocus sequence type; RT, ribotype; WT, wild type.
[b] *tcdC* mutations analyzed were Δ117, 18-bp and 39-bp deletions, and the C184T nucleotide point mutation.
[c] The mutations and genes described are for characterization purposes only, and their presence may not predict antimicrobial resistance in *C. difficile*.

assigned using the software package MLST version 2.16 (6) and the pubMLST *C. difficile* multilocus sequence typing scheme (7). Antimicrobial resistance (AR) genes were identified using a nonredundant combined database of the ResFinder (last updated 1 May 2019 and accessed on 20 May 2019) and ARG-ANNOT (last updated in May 2018 and accessed on 20 May 2019) AR databases; 98% sequence identity and 90% sequence coverage were used. A full description of custom scripts and publicly available tools and versions utilized by QuAISAR-H is available at the GitHub site. Assembled genomes were submitted to the NCBI Prokaryotic Genome Annotation Pipeline for annotation. The Clinical and Laboratory Standards Institute (CLSI) agar dilution method (8) was used to determine MICs for clindamycin, ceftriaxone, meropenem, metronidazole, moxifloxacin, and vancomycin. The results are summarized in Table 1.

The 10 most prevalent RTs collected in 2016 were 027, 106, 002, 014, 020, 015, 056, 054, 019, and 078. All isolates harbored *tcdA* and *tcdB*, and 10/30 (33%) contained *cdtA* and *cdtB*. The six RT027 isolates contained an 18-bp deletion and a deletion at nucleotide position 117 in *tcdC*, resulting in a premature stop codon characteristic of the BI/NAP1/RT027 epidemic strain (9, 10). The two RT078 isolates contained a 39-bp deletion and C184T point mutation in *tcdC*, resulting in a premature stop codon associated with this hypervirulent ribotype (11).

No isolates displayed elevated MICs to vancomycin based on the CLSI epidemiological cutoff value (wild type, $\leq$2 μg/ml; non-wild type, $\geq$4 μg/ml), and all isolates were susceptible to metronidazole (12). No acquired genes conferring resistance to vancomycin or metronidazole were detected. A total of 28 isolates contained genes belonging to the *vanG*-like gene cluster, which is highly conserved among *C. difficile* strains, but their presence is not indicative of vancomycin resistance (13). Recently, investigators suggested that mutations in the regulatory genes *vanS* and *vanR* may be associated with vancomycin resistance in RT027 isolates (14). No RT027 isolates in this panel contained known mutations in *vanS* or *vanR*; however, 3/3 RT002 and 1/3 RT014 (AR-1090) isolates contained the VanS Thr349Ile mutation described by Shen et al. (14) but had a vancomycin MIC of 0.5 μg/ml (Table 1). Five isolates with a clindamycin MIC of $>$16 μg/ml harbored *ermB* genes, which are associated with macrolide-lincosamide-streptogramin B resistance (15, 16). Two of these isolates also contained the *cfrC* gene, which is associated with resistance to phenicols, lincosamides, oxazolidinones, and streptogramins (17, 18). While all isolates contained the wild-type *gyrB* allele, seven with a moxifloxacin MIC of $\geq$8 μg/ml harbored an amino acid substitution (Thr82Ile) in the *gyrA* gene product associated with fluoroquinolone resistance (13).

The "*Clostridioides difficile* EIP 2016" panel represents the first publicly available resource for highly characterized isolates, and additional panels may be included to provide a growing source of contemporary isolates.

Data availability. The *Clostridioides difficile* EIP 2016 panel is available through the CDC & FDA Antibiotic Resistance Isolate Bank (https://wwwn.cdc.gov/ARIsolateBank/). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank, and the first versions are described here. The raw reads were deposited in the Sequence Read Archive (SRA). Assembly and SRA data are available at NCBI under BioProject number PRJNA577141.

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identification only and does not imply endorsement by the U.S. Centers for Disease Control and Prevention, the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

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