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Reference Susceptibility Testing and Genomic Surveillance of *Clostridioides difficile*, United States, 2012–17

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Abstract

Background.—Antimicrobial susceptibility testing (AST) is not routinely performed for *Clostridioides difficile* and data evaluating minimum inhibitory concentrations (MICs) are limited. We performed AST and whole genome sequencing (WGS) for 593 *C. difficile* isolates collected between 2012 and 2017 through the Centers for Disease Control and Prevention's Emerging Infections Program.

Methods.—MICs to 6 antimicrobial agents (ceftriaxone, clindamycin, meropenem, metronidazole, moxifloxacin, and vancomycin) were determined using the reference agar dilution method according to Clinical and Laboratory Standards Institute guidelines. Whole genome sequencing was performed on all isolates to detect the presence of genes or mutations previously associated with resistance.

Results.—Among all isolates, 98.5% displayed a vancomycin MIC ≤2 μg/mL and 97.3% displayed a metronidazole MIC ≤2 μg/mL. Ribotype 027 (RT027) isolates displayed higher vancomycin MICs (MIC_{50}: 2 μg/mL; MIC_{90}: 2 μg/mL) than non-RT027 isolates (MIC_{50}: 0.5 μg/mL; MIC_{90}: 1 μg/mL) ($P < .01$). No *vanA/B* genes were detected. RT027 isolates also showed higher MICs to clindamycin and moxifloxacin and were more likely to harbor associated resistance genes or mutations.

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Potential conflicts of interest.

All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Conclusions.—Elevated MICs to antibiotics used for treatment of *C. difficile* infection were rare, and there was no increase in MICs over time. The lack of *vanA/B* genes or mutations consistently associated with elevated vancomycin MICs suggests there are multifactorial mechanisms of resistance. Ongoing surveillance of *C. difficile* using reference AST and WGS to monitor MIC trends and the presence of antibiotic resistance mechanisms is essential.

Keywords

Clostridioides difficile ; antibiotic resistance; vancomycin; metronidazole; agar dilution

Clostridioides difficile is among the most common causes of healthcare-associated infections in the United States and has emerged as an important cause of diarrhea in those without healthcare exposures [1, 2]. Although the burden of *C. difficile* infections (CDI) in the United States decreased between 2011 and 2017, it remains substantial. In 2017, *C. difficile* was responsible for an estimated 462 100 cases in the United States, including an estimated 224 000 hospitalizations [3].

According to current clinical practice guidelines, fidaxomicin is recommended for the treatment of an initial episode of *C. difficile* infection although vancomycin is an acceptable alternative [4]. Although there is a preference for fidaxomicin over vancomycin, fewer patients may have access to fidaxomicin [5]. For most bacterial infections, antimicrobial susceptibility testing (AST) is performed by the clinical microbiology laboratory to help guide treatment [6]. However, *C. difficile* AST is not typically performed as the infection is usually diagnosed with toxin immunoassays or molecular assays [7]. In addition, the Clinical and Laboratory Standards Institute (CLSI) recommends the agar dilution method for *C. difficile* AST, which is laborious, time consuming, and not practical for clinical laboratories [8].

Because clinical laboratories rarely perform *C. difficile* AST, surveillance studies are important. Combining AST with whole genome sequencing (WGS) is useful to identify strains associated with certain AST patterns. For example, ribotype 027 (RT027) is notable for elevated minimum inhibitory concentrations (MICs) to fluoroquinolones [9, 10] due to mutations in *gyrA* and *gyrB*. Isolates with elevated MICs to vancomycin can be analyzed for acquired resistance genes (eg, *vanA/B*) or mutations in *vanSR*, which regulate $vanG_{Cd}$ expression and have been associated with vancomycin MICs (>2 μg/mL) in RT027 isolates [11].

To describe the epidemiology and molecular characteristics of *C. difficile* in the United States, the Centers for Disease Control and Prevention (CDC) Emerging Infections Program (EIP) conducts active population- and laboratory-based CDI surveillance [3]. In this evaluation, we report agar dilution AST, ribotyping, and WGS data for 593 *C. difficile* isolates collected through EIP surveillance between 2012 and 2017.

METHODS

Surveillance Isolates

Selected counties in 10 US states (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee) have participated in EIP CDI surveillance since 2011. This population-based surveillance activity seeks to capture 100% of CDI cases affecting residents of the catchment area. A case was defined as a positive *C. difficile* toxin or molecular assay on a stool specimen from a surveillance-area resident aged ≥1 year who did not have a positive assay in the previous 8 weeks [3]. Each year, a convenience sample of approximately 1000 specimens from cases across all sites was cultured for *C. difficile* at either the Edward Hines Jr. Veterans Affairs Hospital or the Minnesota Department of Health Public Health Laboratory. Recovered isolates were forwarded to the CDC; between 2012 and 2017, all isolates received underwent polymerase chain reaction (PCR) ribotyping. A subset of 100 isolates per year were selected for AST and WGS. Isolates belonging to one of the 10 most common ribotypes (RTs) (for that year) were eligible to be randomly sampled. The EIP CDI surveillance protocol, including the submission of isolates to CDC, underwent ethical review by CDC and all EIP sites; the protocol was either deemed non-research or was approved by an institutional review board, and a waiver of informed consent was obtained.

Bacterial Growth Conditions and Molecular Typing

Isolates were plated on non-selective CDC anaerobic blood agar with vitamin K, hemin, and 5% sheep blood and incubated anaerobically at 37°C for 24 hours. Strain typing was performed by high-resolution capillary gel-based PCR ribotyping as previously described [12]. Results were analyzed against an in-house library of standard profiles and a 100% similarity threshold to assign RTs (BioNumerics 6.6.11; Applied Maths NV, Belgium).

Agar Dilution Antimicrobial Susceptibility Testing

Isolates underwent AST according to CLSI guidelines for reference agar dilution [8] for the following antibiotics: ceftriaxone, clindamycin, meropenem, metronidazole, moxifloxacin, and vancomycin. *Bacteroides fragilis* ATCC 25285 and *C. difficile* ATCC 700057 were used as quality control strains and were included across all AST panel lots. To evaluate whether MIC differences were statistically significant, Kuiper's test was used to compare the empirical distribution of vancomycin and metronidazole MICs between 2012–2014 and 2015–2017; it was also used to compare the empirical distribution of vancomycin, metronidazole, clindamycin, and moxifloxacin MICs between RT027 and non-RT027 isolates and the empirical distribution of vancomycin MIC for isolates with and without the VanR T115A mutation.

Whole Genome Sequencing

Isolates were sequenced using the MiSeq system (Illumina, San Diego, California, USA) as described by Paulick et al [13]. Data were analyzed using the QuAISAR-H pipeline (run date 25 March 2022, https://github.com/DHQP/QuAISAR_singularity). Antimicrobial resistance (AR) genes were identified using SRST2 [14] and GAMMA [15] against a

non-redundant combined database of the AR databases ResFinder [16], ARG-ANNOT [17], and NCBI's AMRFinder [18] (each accessed 7 May 2021) with the following parameters: minimum of 98% sequence identity and 90% sequence coverage. Isolates with evidence of contamination or with discrepancies between the RT and the WGS-based inferred RT were excluded.

Assemblies were searched for mutations in genes previously associated with elevated MICs to vancomycin (*vanR*, *vanS*) [11] and fluoroquinolones (quinolone-resistance determining region of *gyrA* and *gyrB*) using GAMMA (v1.3) [15]. Wild-type reference *C. difficile* R20291 (GenBank accession: FN545816.1) was used for the *vanR* and *vanS* and *C. difficile* 630 (GenBank accession: AM180355.1) for the *gyrA* and *gyrB* analysis. To detect the presence of plasmid pCD-METRO (GenBank accession CAADHH010000057), reads were mapped with SRST2 (v0.2.0) against 8 pCD-METRO (the plasmid associated with metronidazole resistance) coding sequences [19]. Short-read WGS data for all isolates were submitted to the NCBI Sequence Read Archive under BioProject numbers PRJNA629351 and PRJNA577141 and accession numbers are provided in Supplementary Table 1.

RESULTS

Description of Isolates

Between 2012 and 2017, EIP sites submitted 6964 *C. difficile* isolates to CDC, ranging from 969 in 2016 to 1443 in 2012. Among the 100 isolates selected each year (from among the top 10 RTs), 7 were excluded due to failure to meet WGS quality metrics. This left a set of 593 isolates for analysis, comprising 14 RTs (Table 1); RT027 was most common (23.1%), followed by RT106 (16.9%), RT002 (13.3%), RT020 (11.3%), and RT014 (11.0%). These 5 RTs remained the most common in each study year (2012–2017).

Antimicrobial Susceptibility Testing

The vancomycin MIC_{50} was 0.5 μg/mL and the MIC_{90} was 2 μg/mL for all isolates; 98.5% of isolates had an MIC ≤2 μg/mL (considered wild-type by CLSI and susceptible by the European Committee on Antimicrobial Susceptibility Testing, EUCAST) (Table 2) [20, 21]. The vancomycin MIC distribution did not shift over time ($P = .28$) (Table 3). The RT027 vancomycin MIC distribution was shifted toward higher MICs (MIC_{50}: 2 μg/mL; MIC_{90}: 2 μg/mL) than the non-RT027 distribution (MIC_{50}: 0.5 μg/mL; MIC_{90}:1 μg/mL) ($P < .01$) (Figure 1A, Supplementary Table 2).

The metronidazole MIC_{50} was 0.5 μg/mL, and the MIC_{90} was 2 μg/mL for all isolates; 97.3% of isolates had an MIC ≤2 μg/mL (considered susceptible by EUCAST) (Table 2) [21]. In total, 100% of isolates had an MIC ≤8 μg/mL (considered susceptible by CLSI) [20]. There was a small downward shift in the metronidazole MIC distribution over time ($P < .01$) (Table 3). The RT027 metronidazole MIC distribution was slightly shifted toward higher MICs than the non-RT027 distribution ($P < .01$) (Figure 1B, Supplementary Table 3).

The clindamycin MIC_{50} was 4 μg/mL, and the MIC_{90} was >16 μg/mL (Table 2). The RT027 clindamycin MIC distribution was shifted toward higher MICs than the non-RT027 distribution ($P < .01$) (Figure 1C, Supplementary Table 4). The moxifloxacin MIC_{50} was 2

μg/mL, and the MIC_{90} was >8 μg/mL (Table 2). The RT027 moxifloxacin MIC distribution was shifted toward higher MICs than the non-RT027 distribution ($P < .01$) (Figure 1D, Supplementary Table 5). The ceftriaxone MIC_{50} was 32 μg/mL, and the MIC_{90} was 64 μg/mL (Table 2). The meropenem MIC_{50} and the MIC_{90} were 2 μg/mL (Table 2). The MICs for all isolates and antibiotics tested are included in Supplementary Table 1.

Whole Genome Sequencing

All sequences were analyzed for the presence of *vanA/B*, and none were detected. The $vanG_{Cd}$ gene cluster was detected in 95.8% (568/593) of isolates. The VanR T115A mutation was present in 11% of isolates (65/568) with the $vanG_{Cd}$ cluster [11], and all were RT027. Of the 137 RT027 isolates, 65 (47.4%) harbored the mutation. The vancomycin MIC distribution of the 65 RT027 isolates with the VanR T115 mutation (MIC range:1–4 μg/mL; MIC_{50}: 2 μg/mL; MIC_{90}: 4 μg/mL) was shifted toward slightly higher MICs than the distribution of the 72 RT027 isolates without the VanR T115A mutation (MIC range: 0.5–4 μg/mL; MIC_{50}: 0.5 μg/mL; MIC_{90}: 2 μg/ mL) ($P < .01$). Among the 9 isolates with a vancomycin MIC of 4 μg/mL, all were RT027 and 8 contained the VanR T115A mutation. A VanS T349I amino acid mutation [11] was detected in 20.1% (119/593) of isolates, comprising RT002, RT014, and RT005 with a vancomycin MIC range of 0.5–1 μg/mL. One RT027 isolate with a vancomycin MIC of 2 μg/mL had a S313F VanS amino acid mutation [11]. No isolates contained coding mutations in both VanR and VanS. The pCD-METRO plasmid, which is associated with metronidazole MICs ≥8 μg/mL [19], was not detected in any isolates.

Among the 370 isolates with a clindamycin MIC range of ≤0.25–4 μg/mL, no *erm* or *cfr* genes [22, 23] were detected (Supplementary Table 4). Of the 130 isolates with a clindamycin MIC of 8 μg/mL, 2 harbored *cfrC*, and none contained *erm* genes. In contrast, 84/93 (90.3%) isolates with a clindamycin MIC of >16 μg/mL had an *erm* gene detected, including *ermB* (81 isolates), *ermQ* (2 isolates), and *ermG* (1 isolate); 68 (81.0%) of the 84 *erm* gene-harboring isolates were RT027. A *cfr* gene was present in 38/93 (40.9%) isolates with a clindamycin MIC of >16 μg/mL, including *cfrC* (34 isolates), *cfrB* (2 isolates), and *cfrE* (2 isolates); 29/38 (76.3%) of these isolates were RT027. There were 31 isolates with a clindamycin MIC of >16 μg/mL that had both an *erm* and *cfr* gene and 2 isolates that did not have *erm* or *cfr* (Supplementary Table 4).

No isolates with a moxifloxacin MIC of 1, 2, or 4 μg/mL had amino acid substitutions in GyrA or GyrB. Among the 142 isolates with a moxifloxacin MIC ≥8 μg/mL, the majority (134/142; 94.4%) had a T82I GyrA mutation; 7 of these had both the T82I GyrA and D426N GyrB mutations (Supplementary Table 5). Of the 8 isolates with a moxifloxacin MIC ≥8 μg/mL that lacked the T82I GyrA mutation, 3 had the D426N GyrB mutation, 1 had the R447K mutation, and 4 had no GyrA or GyrB mutations. Although various RTs harbored the T82I GyrA mutation, most (111/134, 82.8%) of these were RT027 (Supplementary Table 5).

DISCUSSION

We characterized 593 *C. difficile* isolates representing 14 different ribotypes collected during 2012–2017 from 10 US sites; 98.5% displayed a vancomycin MIC ≤2 μg/mL and

97.3% displayed a metronidazole MIC ≤2 μg/mL. The MICs for these antibiotics did not increase over time.

Our results are consistent with the findings of several other investigations using reference agar dilution AST on large sets of *C. difficile* isolates over similar time periods in multiple countries [24–27]. In these studies, the vancomycin MIC_{90} has consistently been 2 μg/mL with 93.5% to 98.8% of isolates having an MIC ≤2 μg/mL [24–27]. These results (including ours) are in contrast to those of Darkoh et al who found that between 2012 and 2017, 26% of Houston diarrheal stool samples (114/438) and 67% of Nairobi diarrheal stool samples (66/98) harbored *C. difficile* isolates with a vancomycin MIC ≥4 μg/mL [28]. The differences can likely be explained by the laboratory methods used [29–31]. Our study, and other similar studies, all performed reference agar dilution AST according to the method recommended by CLSI [8, 24–27, 32]. Darkoh et al selected isolates by using *C. difficile* culture media supplemented with antibiotics and then performed AST using gradient diffusion strips and broth microdilution. Broth microdilution is not recommended by CLSI for *C. difficile* AST and vancomycin gradient diffusion strips are not approved by the Food and Drug Administration (FDA) for *C. difficile* [8, 32].

Even for the 9 isolates in this study with a vancomycin MIC >2 μg/mL, the clinical implications are unclear. A recent study demonstrated that elevated metronidazole MICs (defined as ≥1 μg/mL) are associated with an increased risk of treatment failure [33], but there are not similar data available for vancomycin. This may be due to the high fecal concentrations (100 to 1000 times higher than the MIC_{90}) seen with vancomycin [34]. Thus, there are no US FDA or CLSI *C. difficile* breakpoints for vancomycin, although CLSI has established an epidemiologic cutoff value for vancomycin (MIC ≤2 μg/mL is wild-type and MIC ≥4 μg/mL is non-wild-type) [20, 35]. CLSI does have breakpoints for metronidazole (MIC ≤8 μg/mL is susceptible, MIC =16 μg/mL is intermediate, and MIC ≥32 μg/mL is resistant); however, these breakpoints apply to all anaerobes and are not specific to *C. difficile* [20]. The European Committee on Antimicrobial Susceptibility Testing provides *C. difficile* breakpoints for metronidazole and vancomycin. Both are based on epidemiological cutoff values where MIC ≤2 μg/mL is susceptible and MIC >2 μg/mL is resistant [21].

In this study, no *vanA/B* genes were detected, even among the 9 RT027 isolates with vancomycin MICs of 4 μg/mL. To further investigate potential genetic mechanisms associated with elevated vancomycin MICs, an analysis of the $vanG_{Cd}$ operon was conducted. Expression of the $vanG_{Cd}$ operon leads to modification of peptidoglycan precursors, resulting in reduced vancomycin cell wall binding [11]. The $vanG_{Cd}$ gene cluster was common, with 95.8% of isolates containing the operon, which is higher than a previous estimate of 85% [36]. Although the $vanG_{Cd}$ operon has been confirmed to be functional and inducible in *C. difficile*, isolates containing this operon display MICs ≤2 μg/mL [36]. However, Shen et al recently described mutations in the $vanG_{Cd}$ regulatory genes *vanS* and *vanR* in RT027 isolates that resulted in constitutive expression of $vanG_{Cd}$, and elevated vancomycin MICs (4–8 μg/mL and 8–16 μg/mL for isolates with *vanR* and *vanS* mutations, respectively) [11]. One of these mutations, VanR T115A, was detected in 47.4% of the RT027 isolates included in this study, and these isolates displayed significantly higher vancomycin MICs than RT027 isolates without the mutation. However, the presence

of VanR T115A was not always associated with elevated vancomycin MICs as only 8/65 RT027 isolates with the mutation displayed a vancomycin MIC of 4 μg/mL. Because 87.7% of isolates with the T115A mutation had an MIC ≤2 μg/mL, this mutation may represent a common variant in the RT027 population. A mutation in VanS (T349I) previously associated with elevated vancomycin MICs (8–16 μg/mL) in RT027 [11] was also detected among our study isolates including RT002, RT014, and RT005. However, none of these isolates showed an elevated vancomycin MIC (range: 0.5–1 μg/mL), which brings into question the significance of this mutation, at least in these RTs. Overall, these results suggest that the genetic mechanisms of vancomycin resistance may be multifactorial.

In the present study, RT027 isolates were found to display higher MICs to vancomycin, metronidazole, clindamycin, and moxifloxacin and were more likely to harbor associated AR genes or mutations. Among isolates with a clindamycin MIC >16 μg/mL, 76.3% were RT027% and 87.1% harbored the *ermB* gene, a macrolide-lincosamide-streptogramin B (MLS_B) resistance determinant [37]. In addition, 40.9% of isolates with a clindamycin MIC >16 μg/mL carried a *cfr*-like gene encoding a 23S rRNA methyltransferase. The 3 *cfr*-like genes identified have been associated with elevated MICs to linezolid and have been found on various chromosomally encoded mobile genetic elements [23]. A significant proportion (22.9%) of study isolates had elevated moxifloxacin MICs (>8 μg/mL), and 81.6% of these isolates were RT027 and contained the GyrA T82I mutation. Although moxifloxacin is not indicated for treatment of CDI, it may play a role in selecting specific strains, especially RT027 [38]. However, 7 additional ribotypes showed elevated moxifloxacin MICs and contained GyrA or GyrB mutations (Supplementary Table 5), highlighting a potential role for fluoroquinolones in the selection of other strain types besides RT027. Although no isolates were found to harbor the recently described pCD-METRO, the single isolate with an elevated metronidazole MIC of 8 μg/mL was RT027. Our results demonstrate that the epidemic hypervirulent RT027 strain remains the most likely to harbor fluoroquinolone and clindamycin resistance determinants in US patients. This observed association of RT027 isolates with increased MICs to multiple antibiotics is consistent with surveillance of antibiotic resistance trends in Europe [39].

The major strength of our study was that we used the reference agar dilution method to investigate the susceptibility of a large number of isolates collected over several years and across multiple, geographically diverse US regions. In addition, WGS was performed on all isolates to provide a comprehensive review of acquired resistance mechanisms present among *C. difficile* isolates.

This study also has limitations. First, the isolates sent to CDC were a convenience sample and may not be representative of the RTs at each site. The isolates were collected between 2012 and 2017 and were selected among the most frequently occurring US ribotypes. In addition, the isolates in the 10 EIP sites that participated in this study may not be representative of the isolates in the United States. Second, we did not perform repeat AST for isolates with elevated MICs to vancomycin (or any isolates) and acknowledge that, upon repeat testing, the MIC for an isolate may fall within one doubling dilution of the observed MIC. Finally, we did not evaluate susceptibility to fidaxomicin because the antibiotic was

not available to CDC at the time of testing; however, fidaxomicin will be included in future AST of EIP surveillance isolates.

In summary, elevated MICs to clinically relevant drugs, such as vancomycin and metronidazole, are rare among *C. difficile* collected in the areas participating in EIP CDI surveillance; MICs for these drugs were not increasing over time. The epidemic hypervirulent strain RT027 was associated with increased MICs to multiple antibiotics. Continued surveillance with reference AST and WGS will be crucial to detect the emergence of *C. difficile* isolates with increased MICs to vancomycin and metronidazole.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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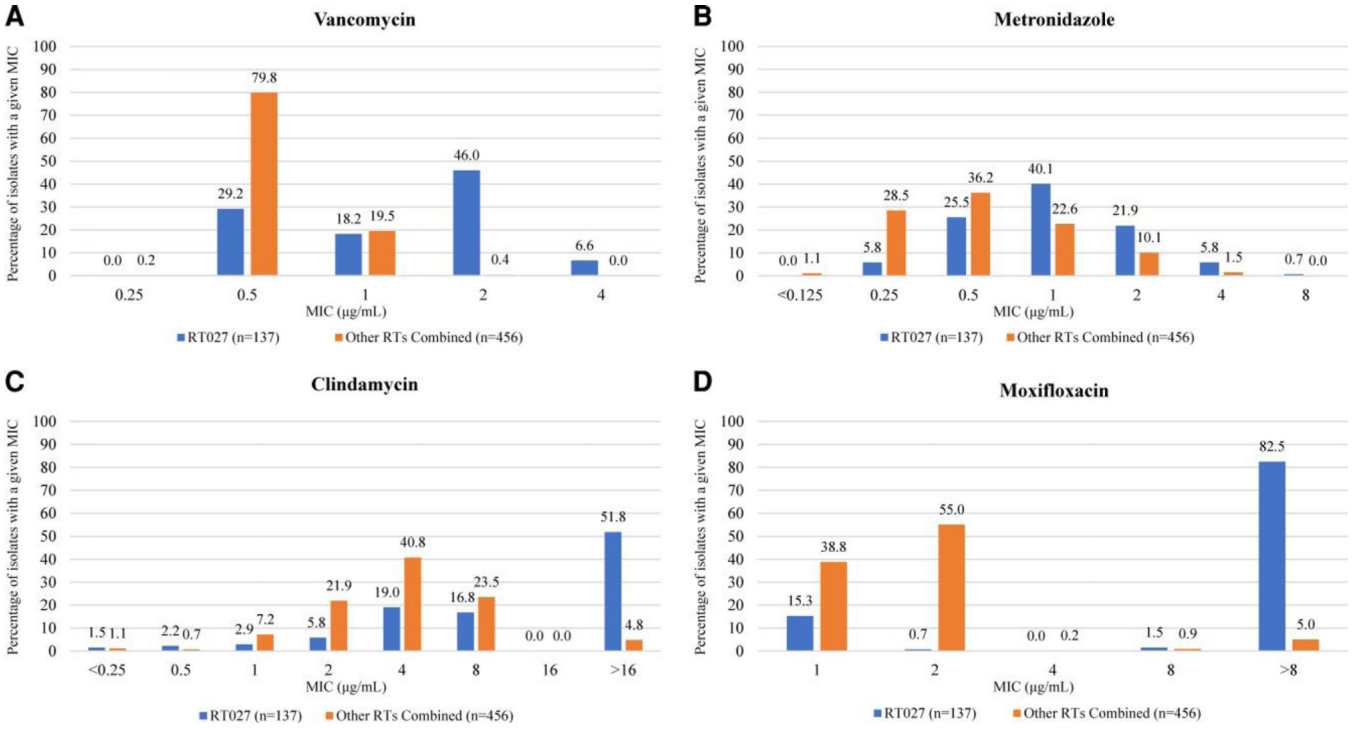

Figure 1.

The MIC distribution of RT027 Emerging Infections Program 2012–2017 isolates is compared to the non-RT027 isolates for vancomycin (*A*), metronidazole (*B*), clindamycin (*C*), and moxifloxacin (*D*). Abbreviation: MIC, minimum inhibitory concentration.

Table 1.

Description of *C. difficile* Emerging Infections Program 2012–2017 Isolates for Which Antimicrobial Susceptibility Testing and Whole Genome Sequencing Was Performed (N = 593)

Ribotype	Total Number of Isolates	Years Collected
001_072[a]	16 (2.7%)	2012–15
002	79 (13.3%)	2012–17
005	7 (1.2%)	2015
014	65 (11.0%)	2012–17
015	30 (5.1%)	2012–17
017	2 (0.3%)	2013
019	5 (0.8%)	2016
020	67 (11.3%)	2012–17
027	137 (23.1%)	2012–17
054	23 (3.9%)	2012, 2014–17
056	31 (5.2%)	2012–17
076	13 (2.2%)	2014, 2017
078	18 (3.0%)	2012–13, 2016–17
106	100 (16.9%)	2012–17
All	593 (100%)	2012–17

[a]RT001 and RT072 are indistinguishable by high-resolution capillary gel-based polymerase chain reaction (PCR) ribotyping.

Table 2.

Summary of Antimicrobial Susceptibility Testing Data for *C. difficile* Emerging Infections Program 2012–2017 Isolates (N = 593)

	Number of Isolates (Cumulative %) Inhibited at a MIC μg/mL) of:											
Antimicrobial agent[a]	≤0.125	0.25 (or ≤0.25)[b]	0.5	1	2	4	8	16 (or >8)[c]	32 (or >16)[d]	64	128	>128
Ceftriaxone	...	...	...	...	...	...	...	57 (9.6%)	361 (70.5%)	123(91.2%)	42(98.3%)	10(100%)
Clindamycin	...	7[a] (1.2%)	6 (2.2%)	37 (8.4%)	108 (26.6%)	212 (62.4%)	130 (84.3%)	...	93[c](100%)	...	...	...
Meropenem	...	...	3 (0.5%)	142 (24.5%)	433 (97.5%)	15 (100%)	...	...	...	...	...	...
Metronidazole	5 (0.8%)	138(24.1%)	200 (57.8%)	158 (84.5%)	76 (97.3%)	15 (99.8%)	1 (100%)	...	...	...	...	...
Moxifloxacin	...	...	...	198 (33.4%)	252 (75.9%)	1 (76.1%)	6 (77.1%)	136[b] (100%)	...	...	...	...
Vancomycin	...	1 (0.2%)	404 (68.3%)	114(87.5%)	65 (98.5%)	9 (100%)	...	...	...	...	...	...

Abbreviation: MIC, minimum inhibitory concentration.

[a]Tested drug ranges: Ceftriaxone: 8–128 μg/mL, Clindamycin 0.25–16 μg/mL, Meropenem 0.25–16 μg/mL, Metronidazole 0.125–32 μg/mL, Moxifloxacin 0.5–8 μg/mL, and Vancomycin 0.125 to 16 μg/mL.

[b]Seven isolates had a clindamycin MIC result of ≤0.25 μg/mL.

[c]In total, 136 isolates had a moxifloxacin MIC result of >8 μg/mL.

[d]In total, 93 isolates had a clindamycin MIC result of >16 μg/mL.

Table 3.

Summary of *C. difficile* Emerging Infections Program 2012–2017 Metronidazole and Vancomycin Minimum Inhibitory Concentration (MIC) Data Over Time (N = 593)

	Number of isolates	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	MIC Range (μg/mL)	MIC ≤2 μg/mL[a]
Metronidazole					
2012	98	1	2	≤0.125–8	94.9%
2013	99	0.5	1	0.25–4	99.0%
2014	100	1	2	0.25–4	99.0%
2015	97	1	2	0.5–4	90.7%
2016	99	0.5	1	≤0.125–1	100%
2017	100	0.25	0.5	≤0.125–0.5	100%
Total	593	0.5	2	≤0.125–8	97.3%
Vancomycin					
2012	98	0.5	1	0.5–4	98.0%
2013	99	0.5	2	0.5–2	100%
2014	100	0.5	1	0.5–4	98.0%
2015	97	0.5	2	0.5–4	99.0%
2016	99	0.5	2	0.25–2	100%
2017	100	0.5	2	0.5–4	96%
Total	593	0.5	2	0.25–4	98.5%

Abbreviation: MIC, minimum inhibitory concentration.

[a]MIC≤2 μg/mL is the susceptible breakpoint for both metronidazole and vancomycin according to EUCAST [21] and is the epidemiological cutoff value for vancomycin according to CLSI [20].

The metronidazole breakpoint according to CLSI is ≤8 μg/mL; according to this breakpoint, all tested isolates were susceptible to metronidazole.