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Background: The epidemic NAP1/027 *Clostridioides difficile* strain (MLST1, ST1) that emerged in the mid-2000 is on the decline. The current distribution of *C. difficile* strain types and their transmission dynamics are poorly defined. We performed whole-genome sequencing (WGS) of *C. difficile* isolates in 2 regions to identify the predominant multilocus sequence types (MLSTs) in community- and healthcare-associated cases and potential transmission between cases using whole-genome single-nucleotide polymorphism (SNP) analysis. **Methods:** Isolates were collected through the CDC Emerging Infections Program population-based surveillance for *C. difficile* infections (CDI) for 3 months between 2016 and 2017 in 5 Minnesota counties and 1 New York county. Isolates were limited to incident cases (CDI in a county resident with no positive *C. difficile* test in the preceding 8 weeks). Cases were classified as healthcare associated (HA-CDI) or community associated (CA-CDI) based on healthcare exposures as previously described. WGS was performed on an Illumina Miseq. The CFSAN (FDA) pipeline was used to compute whole-genome SNPs, SPAdes was used for assembly, and MLST was assigned according to www.pubmlst.org. **Results:** Of 431 isolates, 269 originated from New York and 162 from Minnesota; 203 cases were classified as CA-CDI and 221 as HA-CDI. The proportion of CA-CDI cases was higher in Minnesota than in New York: 62% vs 38%. The predominant MLSTs across both sites were ST42 (9%), ST8 (8%), and ST2 (8%). MLSTs more frequently encountered in HA-CDI than CA-CDI included ST1 (note that this ST includes PCR Ribotype 027; 76% HA-CDI), ST53 (84% HA-CDI), and ST43 (80% HA-CDI). In contrast, ST110 (63% CA-CDI) and ST3 (67% CA-CDI) were more commonly isolated from CA-CDI cases. ST1 accounted for 7.6% of circulating strains and was more common in New York than Minnesota (10% vs 3%) and was concentrated among New York HA-CDI cases. Also, 412 isolates (1 per patient) were included in the final whole-genome SNP analysis. Of these, only 12 pairs were separated by 0–3 SNPs, indicating potential transmission, and most involved HA-CDI cases. ST1, ST17, and ST46 accounted for 8 of 12 pairs, with ST17 and ST46 potentially forming small clusters. **Conclusions:** This analysis provides a snapshot of the current genomic epidemiology of *C. difficile* across 2 geographically and epidemiologically distinct regions of the United States and supports other studies suggesting that the role of direct transmission in the spread of CDI may be limited.

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Genomic Epidemiology of *Clostridioides difficile* Sequence Types 1 and 2 Across Three US Medical Centers

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Background: *Clostridioides difficile* is a toxin-producing bacterium that is the foremost cause of healthcare-associated diarrhea in the United States. Recent epidemiologic and genomic evidence indicates that divergent *C. difficile* strains have varying propensities for transmission within healthcare settings. We investigated whether and how these differences are reflected in the genomic epidemiology of 2 common *C. difficile* strains—sequence type (ST) 1 (analogous to Ribotype 027) and ST2 (associated with Ribotypes 014/020)—across 3 geographically distinct US medical centers. **Methods:** Between 2011 and 2017, a convenience sample of ST1 and ST2 *C. difficile* clinical isolates were collected from 3 US sites: The University of Michigan Medical Center, Texas Medical Center Hospitals, and Memorial Sloan Kettering Cancer Center. Isolates underwent whole-genome sequencing and *in silico* multilocus sequence typing to verify strain types. Sequences were mapped to ST1 and ST2 reference genomes and single nucleotide variants (SNVs) were identified, filtered, and used to construct pairwise SNV distance matrices. A range of pairwise SNV distance thresholds were applied to assess genetic linkages consistent with recent transmission within ST1 compared to within ST2. Proportions of genetically linked isolates were compared using χ^2 tests. **Results:** We identified 200 ST1 and 188 ST2 isolates across the 3 collection sites. Overall, ST2 was more genetically diverse than ST1 (pairwise SNV distance range, 0–156 SNVs and 0–78 SNVs, respectively). ST2 isolates displayed significantly less evidence of recent transmission: 10 ST2 isolates (5.3%) were within 2 SNVs of another isolate compared to 88 (44%) ST1 isolates ($P \leq .001$) (Fig. 1). As the SNV threshold increased to 5 and 10 SNVs, this trend was maintained (all $P < .001$). ST2 isolates were also more likely to be genetically linked to an isolate from a different collection site than ST1 isolates. Among isolates with genetic links to at least 1 other isolate at the 5 SNV and 10 SNV thresholds, 21 of 37 and 74 of 89 ST2 isolates (57%, 83%) were linked to an isolate from a different collection site, compared to 2 of 88 and 48 of 157 ST1 isolates (2% and 31%, respectively; both $P < .001$). **Conclusions:** Compared to *C. difficile* ST1 isolates, ST2 isolates displayed less evidence of recent healthcare transmission and were more likely to be genetically linked to isolates from divergent collection sites. Interpreting genetic linkages among *C. difficile* isolates requires an understanding of regional and strain-specific genetic diversity to avoid misattribution of genetic linkages to recent transmission.

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