validate the EHR search we reviewed these records. A total of 135 (73.4%) patients had been treated for LTBI and 28 (15.2%) had been or were being treated for active pulmonary TB or an atypical mycobacterial infection. The remaining 23 (11.4%) had been prescribed isoniazid or rifampin for another reason, had previously been treated for LTBI and isoniazid or rifampin was documented as a historical medication, or refused treatment. No patients had been prescribed rifapentine. Of those who began treatment for LTBI, 101 (74.8%) completed or were still undergoing treatment at the time of the study, 5 (3.7%) stopped treatment, and treatment completion was unknown for 29 (21.6%).

In our tertiary/quaternary medical center, which serves a large population of foreign-born patients, we found self-reported nationality and preferred language to be a good proxy for foreign-born persons and others who meet the US Preventive Service Task Force and Centers for Disease Control and Prevention guidelines for LTBI screening. However, our single-center study is in a unique setting and so might not reflect findings in other settings. Our proposed screening strategy might miss persons who prefer speaking English but would otherwise meet criteria for LTBI screening. This study identified missed opportunities for screening and diagnosis of LTBI among foreign-born persons; of those who had a recent diagnosis of LTBI, most were successfully treated. Improved LTBI screening, possibly with the use of routine EHR tools, is needed to end the global TB epidemic.

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References

1. World Health Organization. The End TB Strategy [cited 2019 Sep 5]. https://www.who.int/tb/post2015_strategy
2. Bibbins-Domingo K, Grossman DC, Curry SJ, Bauman L, Davidson KW, Epling JW Jr, et al.; US Preventive Services Task Force. Screening for latent tuberculosis infection in adults: US Preventive Services Task Force recommendation statement. JAMA. 2016;316:962–9. https://doi.org/10.1001/jama.2016.11046
3. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64:111–5. https://doi.org/10.1093/cid/ciw778
4. Centers for Disease Control and Prevention. Latent TB infection in the United States [cited 2019 Sep 5]. https://www.cdc.gov/tb/statistics/ltbi.htm
5. San Diego County Department of Public Health. County of San Diego Tuberculosis Control Program. 2015 fact sheet [cited 2020 Sep 5]. https://www.sandiegocounty.gov/content/dam/sdc/hhsa/programs/phs/tuberculosis_control_program/Factsheet%202015.pdf
6. Centers for Disease Control and Prevention. Latent Tuberculosis Infection: A guide for primary health care providers [cited 2020 Sep 5]. https://www.cdc.gov/tb/publications/ltbi/appendixb.htm

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Putative Conjugative Plasmids with *tcdB* and *cdtAB* Genes in *Clostridioides difficile*

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The major toxins of *Clostridioides difficile* (TcdA, TcdB, CDT) are chromosomally encoded in nearly all known strains. Following up on previous findings, we identified 5 examples of a family of putative conjugative plasmids with *tcdB* and *cdtAB* in clinical *C. difficile* isolates from multilocus sequence typing clades C-I, 2, and 4.

Clostridioides difficile spores may differentiate in the colon of susceptible humans into vegetative cells and release 1 or 2 large clostridial cytotoxins (TcdA, TcdB) or a binary toxin with ADP-ribosyltransferase activity (CDT), or both, to cause colitis and diarrhea (*1*). When present, genes for TcdA, TcdB, and CDT are almost without exception encoded by 2 separate chromosomal loci known as PaLoc and CdtLoc (*2*). Recent discovery of clade C-I strains SA10-050 and

CD10-165 in France (*3*) and HSJD-312 and HMX-152 in Costa Rica (*4*) challenged this paradigm, as these strains carry a monotoxin *tcdB*$^{+}$ PaLoc next to a full CdtLoc on extrachromosomal molecules that resemble conjugative plasmids (*4*).

The Anaerobic Bacteriology Research Laboratory (LIBA) has been isolating and typing *C. difficile* in Costa Rica for nearly a decade and thereby generated an isolate collection with >800 records. We searched mobile genetic elements (MGEs) among whole-genome sequences from 150 of those bacteria, leading to the discovery of 5 new *tcdA*$^{-}$/*tcdB*$^{+}$/*cdtAB*$^{+}$ putative plasmids among isolates that were cultivated from loose fecal samples of patients under clinical suspicion for *C. difficile* infections (CDIs): LIBA-6656, LIBA-7194, LIBA-7602, LIBA-7678, and LIBA-7697. These materials were collected at 3 hospitals located within a 78.5 km^2 area in 2013 (LIBA-6656), 2016 (LIBA-7194), 2017 (LIBA-7602), and 2018 (LIBA-7678, LIBA-7697). Raw sequencing data can be retrieved from the European Nucleotide Archive (https://www.ebi.ac.uk/ena; LIBA-6656, run ERR467623) or from the MicrobesNG platform (https://microbesng.com/portal/projects/FB43968C-E9EF-4270-9D1A-054457CC9B54).

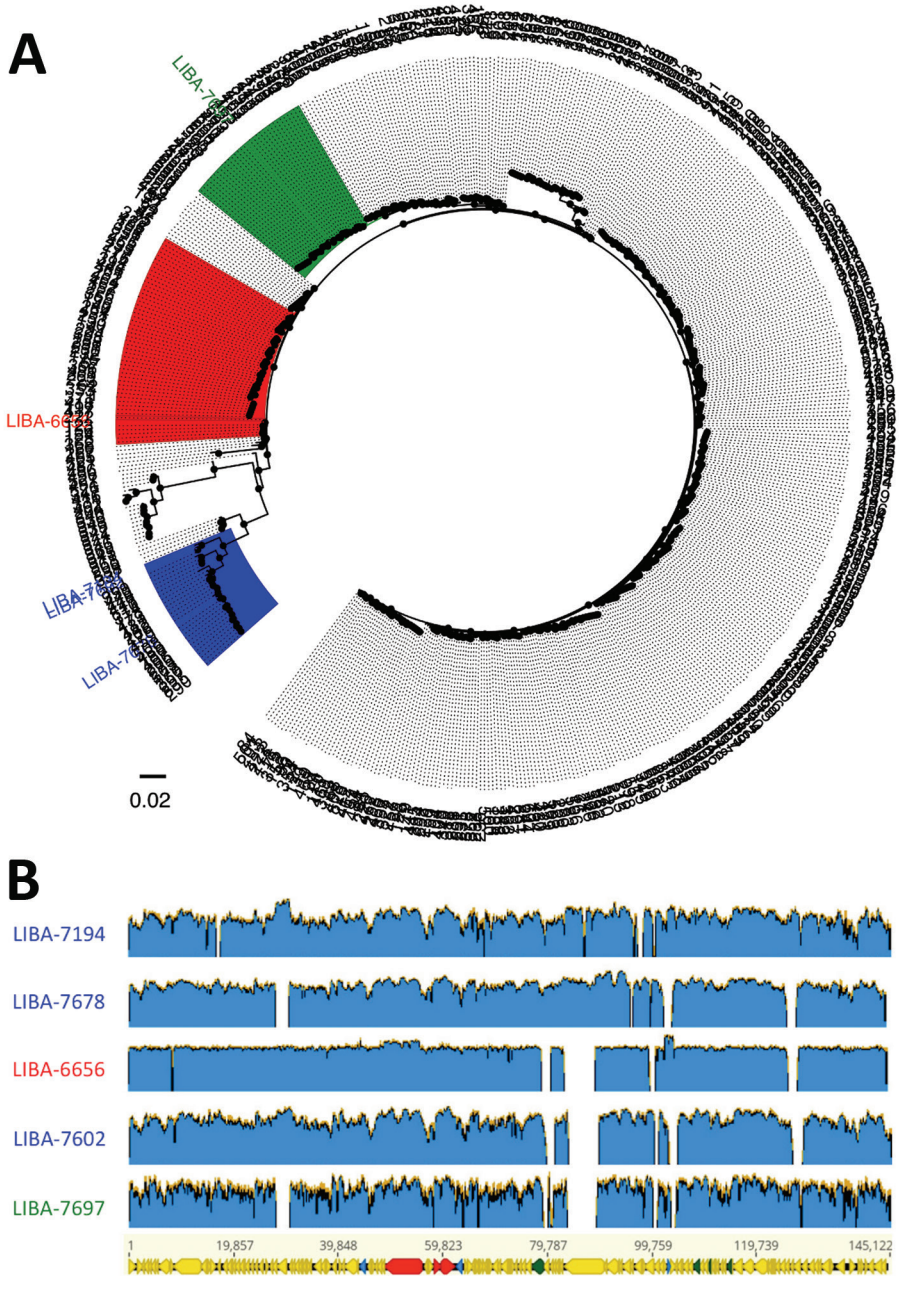

Figure. Multilocus sequence typing–based classification (A) and diversity of extrachromosomal circular sequences (B) of *Clostridioides difficile* strains with plasmid-encoded toxins. A) FastTree (http://www.microbesonline.org/fasttree) phylogenic tree derived from a MUSCLE (http://www.drive5.com/muscle) alignment of concatenated multilocus sequence typing alleles from all *C. difficile* sequence types deposited in the PubMLST database (https://pubmlst.org). Tip labels represent sequence types or strain names. Strains from clade C-I are highlighted in blue, from clade 2 in red, and from clade 4 in green. B) This graphic shows short reads from strains LIBA-7194, LIBA-7678, LIBA-7602 (clade C-I, blue), LIBA-6656 (clade 2, red), and LIBA-7697 (clade 4, green) mapped to the plasmid sequence of strain HSJD-312, which was obtained through hybrid PacBio (Pacific Biosciences, https://www.pacb.com) and Illumina (Illumina, https://www.illumina.com) sequencing and therefore used as a reference (145.1 kb, bottom). Arrows in the reference sequence represent annotated coding sequences. Genes for toxins are in red; for transposases, integrases, and recombinases are in blue, and for proteins from a putative conjugation machinery are in green.

A tree of aligned, concatenated, multilocus sequence typing allele combinations revealed that the new plasmid sequences were present in isolates assigned to clade C-I (LIBA-7194, LIBA-7602, LIBA-7678), clade 2 (LIBA-6656), and clade 4 (LIBA-7697) (Figure 1, panel A). This unexpected result expands the host range of this type of MGE to include *C. difficile* clades more commonly associated with human hosts. The 3 clade C-I strains were different, as confirmed by pairwise estimates of genomic MinHash (min-wise independent permutations locality sensitive hashing scheme) distances (0.022–0.049) calculated with MASH (https://mash.readthedocs.io/en/latest/index.html) and average nucleotide identities (94.15%–97.45%) calculated with FastANI (https://github.com/ParBLiSS/FastANI). Genome similarity showed a tendency to decrease with time (data not shown), suggesting that clade C-I strains are evolving.

Although our plasmid assemblies are awaiting confirmation by long-read sequencing, the size of 3 of the reconstructed plasmids (139.2–147.7 kb) closely matches that of known *C. difficile* toxin plasmids, such as pHSJD-312 (145.1 kb) (*4*). The toxin contigs of LIBA-7697 (53.2 kb) and LIBA-7194 (228.8 kb) were fragmented or likely misassembled, respectively. Read mapping to a high-quality hybrid assembly of pHSJD-312 showed 53.6%–93.7% identical sites in the alignment and 92%–98% reference sequence coverage, indicating that the new toxin plasmids are not the same molecule (Figure 1, panel B). We corroborated this result with a Panaroo (https://github.com/gtonkinhill/panaroo) pangenome analysis of pHSJD-312 and the plasmid sequences found in LIBA-6656, LIBA-7602, and LIBA-7678, because it classified only 135 (89%) of 152 genes as conserved. This core genome included toxin loci, agr loci, and potential conjugation systems. In contrast, mapping gaps corresponded to putative virulence factors (i.e., lectin-binding or cell wall–binding proteins), hypothetical proteins, and MGEs, such as class 2 introns and transposases (Figure 1, panel B). These findings imply that this group of chimeric molecules is undergoing nonhomologous recombination.

The MGE-associated *tcdB* sequence of LIBA-6656 (clade 2) could not be fully assembled. In the remaining 4 strains, this gene was highly conserved (99%–100% protein sequence identity) and expected to encode variant TcdBs that would cause a *Clostridium sordellii*–like cytopathic effect. Besides its plasmid-borne *tcdB*, LIBA-6656 carries a different *tcdB* allele on a chromosomal PaLoc. The contribution of each of these *tcdB* alleles to infection is unclear at this time. Yet, the coexistence of 2 PaLocs within a host is compatible with the suggested transition from ancient monotoxin PaLocs to modern bitoxin PaLocs (*3*). We also noted a high level of sequence identity for *cdtA* (≥99%) and *cdtB* (≥98%) in all 5 putative plasmids. However, it is difficult with such a small dataset to conclude whether the noted conservation of toxin gene sequences reflects stable coevolution or only the short evolutionary time after acquisition.

As previously seen in other clade C-I toxin plasmids, the toxin genes of the new putative plasmids are flanked by genes for a transposase and an integrase (*4*). Furthermore, we identified their PaLocs as lateral gene transfer events using Alien_Hunter software (Sanger Institute, https://www.sanger.ac.uk). Additional elements from this group of MGEs lack toxin genes (*4*), indicating that they are gained through lateral gene transfer.

Three of the 5 isolates that host new toxin plasmids would have remained undetected if we had not attempted *C. difficile* cultivation from TcdB$^-$ fecal samples or sequencing for isolates with negative results for *tcdC* and *tcdA* (LIBA-7194, LIBA-7602, LIBA-7678). We therefore anticipate that the frequency of *C. difficile* isolates with toxin plasmids has been underestimated and recommend that current diagnostic procedures be refined. Moreover, our results open avenues to explore whether similar plasmids are present in species other than *C. difficile* and are implicated in undiagnosed cases of antibiotic-associated diarrhea.

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References

1. Balsells E, Shi T, Leese C, Lyell I, Burrows J, Wiuff C, et al. Global burden of *Clostridium difficile* infections: a systematic review and meta-analysis. J Glob Health. 2019;9:010407. https://doi.org/10.7189/jogh.09.010407

2. Knight DR, Elliott B, Chang BJ, Perkins TT, Riley TV. Diversity and evolution in the genome of *Clostridium difficile*. Clin Microbiol Rev. 2015;28:721–41. https://doi.org/10.1128/CMR.00127-14
3. Monot M, Eckert C, Lemire A, Hamiot A, Dubois T, Tessier C, et al. *Clostridium difficile*: new insights into the evolution of the pathogenicity locus. Sci Rep. 2015;5:15023. https://doi.org/10.1038/srep15023
4. Ramírez-Vargas G, López-Ureña D, Badilla A, Orozco-Aguilar J, Murillo T, Rojas P, et al. Novel Clade C-I *Clostridium difficile* strains escape diagnostic tests, differ in pathogenicity potential and carry toxins on extrachromosomal elements. Sci Rep. 2018;8:13951. https://doi.org/10.1038/s41598-018-32390-6

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Information-Accessing Behavior during Zika Virus Outbreak, United States, 2016

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We used latent class analysis to examine Zika virus–related information-accessing behavior of US residents during the 2016 international outbreak. We characterized 3 classes of information-accessing behavior patterns: universalists, media seekers, and passive recipients. Understanding these patterns is crucial to planning risk communication during an emerging health threat.

During the past 15 years, new media platforms have emerged as routine channels of health communication. Little is known about how persons navigate this dynamic and complex information landscape, especially during an emerging health threat with little scientific certainty and few or no medical countermeasures (*1*,*2*). The 2016 Zika virus outbreak provides for an examination of how people interact with this dynamic information landscape. As scientific understanding of the virus evolved, so did Zika risk communication strategies. Previous reports have identified public sources of Zika information but have not considered the public's information-accessing behavior (*3*,*4*). We used latent class analysis (LCA) to characterize and differentiate types of information-accessing behavior and identify how these behavioral patterns shifted during the 2016 Zika virus outbreak.

LCA identifies clusters within the population on the basis of participants' responses to observed variables (*5*,*6*). We collected and pooled data from 3 representative samples of US households drawn from fully replicated, single-stage, random-digit dialing samples of households supplemented by lists of randomly generated cell phone numbers. The survey had a 4%–6% response rate. We conducted the surveys in April–May (1,233 participants), July–August (1,231 participants), and October–November (1,234 participants) of 2016.

The survey analyzed access to 6 categories of information sources: news (online or print); television or radio; social media, such as Facebook, YouTube, Reddit, or other apps; personal physician; government agencies; and friends, family, or co-workers. We used these data to form 6 binary variables indicating access to each category of information source. We then used these variables to determine 3 classes of information-accessing behavior.

In accordance with the best practices suggested by Nylund et al. (*7*), we used 6 criteria to determine the optimal number of classes (Appendix, https://wwwnc.cdc.gov/EID/article/26/9/19-1519-App1.pdf). New York University's Institutional Review Board approved this research.

Our LCA results suggested that information-accessing behaviors could be grouped into 3 distinct classes: universalists, media seekers, and passive recipients. We sorted each participant into a class on the basis of the number of sources he or she had accessed (Figure). Class 1 comprised universalists, that is, participants who actively accessed information from all sources included in the survey. Class 2 comprised media seekers, that is, participants who primarily accessed information from mass media. Class 3 comprised passive recipients of information; these participants accessed the fewest number of sources and had the highest probability of seeking information from broadcast media. Class membership was not necessarily static; an individual participant might exhibit different information-accessing behaviors at different time points within the Zika outbreak.

The acquisition patterns of Zika information shifted across time. At the first time point (April–May 2016), universalists constituted 23.0% of the US population, media seekers 20.7%, and passive recipients 54.3%. At the second time point (July–August 2016),