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Genomic Determination of Relative Risks for *Clostridioides difficile* Infection From Asymptomatic Carriage in Intensive Care Unit Patients

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Background. *Clostridioides difficile* infections (CDIs) are among the most prevalent hospital-associated infections (HAIs), particularly for intensive care unit (ICU) patients. The risks for developing active CDI from asymptomatic carriage of *C. difficile* are not well understood.

Methods. We identified asymptomatic *C. difficile* carriage among 1897 ICU patients using rectal swabs from an existing ICU vancomycin-resistant enterococci (VRE) surveillance program. *C. difficile* isolates from VRE swabs, and from *C. difficile*–positive stool samples, were genome sequenced. Spatial-temporal data from hospital records assessed genomically identified clusters for potential transmission events.

Results. Genomic analyses identified a diverse set of strains in infected patients and asymptomatic carriers. A total of 7.4% of ICU patients asymptomatically carried *C. difficile*; 69% of isolates carried an intact toxin locus. In contrast, 96% of *C. difficile* stool isolates were toxin encoding. CDI rates in asymptomatic carriers of toxin-encoding strains were 5.3% versus 0.57% in noncarriers. The relative risk for CDI with asymptomatic carriage of a toxin-encoding strain was 9.32 (95% confidence interval, 3.25–26.7). Genomic identification of clonal clusters supported analyses for asymptomatic transmission events, with spatial-temporal overlaps identified in 13 of 28 cases.

Conclusions. Our studies provide the first genomically confirmed assessments of CDI relative risk from asymptomatic carriage of toxin-encoding strains and highlight the complex dynamics of asymptomatic transmission in ICUs. Asymptomatic carriers are an active reservoir of *C. difficile* in the nosocomial environment. *C. difficile* screening can be implemented within existing HAI surveillance programs and has the potential to support infection-control efforts against this pathogen.

Keywords. *Clostridioides difficile*; ICU; asymptomatic carriage; genomic epidemiology; screening.

Clostridioides difficile infection (CDI) is the most prevalent healthcare-associated pathogen [1], costing more than $5 billion annually in the United States, from more than 500 000 infections and more than 29 000 deaths [2]. Risks for CDI include chronic contact with healthcare systems, use of broad-spectrum antibiotics, and underlying medical conditions including inflammatory bowel disease or prior CDI [3]. Asymptomatic carriage has also been linked to increased risks for CDI [4]. *Clostridioides difficile* infection occurs from pathogen-released toxins, particularly toxins A and B, encoded by its pathogenicity locus [5]. A third toxin, binary toxin (CDT), is associated with more severe disease [6].

Asymptomatic carriers are thought to contribute to CDI in healthcare facilities [7–11], but their activity as pathogen reservoirs, and contributions to their own risks for CDI, are poorly defined [12–14]. One interventional study that placed asymptomatic carriers on contact precautions successfully decreased CDI [15, 16]. Healthcare workers have also been identified as potential carriers of toxin-encoding strains, but at rates reflective of the general population [17–19].

We hypothesized that asymptomatic carriage of *C. difficile* increased the risks for infections, and that carriers may provide an undetected reservoir for *C. difficile* transmission. To evaluate this hypothesis, we undertook intensive care unit (ICU) surveillance for *C. difficile* over an 8-month period. Analyses validated the sensitivity of a culture-based screening method for *C. difficile* using rectal swabs collected for an existing ICU screening program for vancomycin-resistant enterococci (VRE) [4, 13, 20, 21]. *Clostridioide difficile* was also isolated from toxin-positive stool samples collected from all hospitalized patients to compare genomic findings in asymptomatic carriers with symptomatic patients. Integrated genomic and epidemiologic analyses identified

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strain dynamics and supported the development of a platform leveraging clinical infrastructure and nationally available resources to improve surveillance efforts for *C. difficile* [22].

METHODS

Study Protocol

Brigham and Women's Hospital (BWH) in Boston, Massachusetts, is a 793-bed hospital providing care to more than 600 000 patients per year, including more than 36 000 inpatient admissions. The hospital includes multiple ICUs. The study was carried out under Institutional Review Board protocol 2011-P-002883 (L. B., Partners Healthcare), which allows access to discarded clinical samples and medical records in support of hospital infection-control investigations and clinical laboratory assay development. The Crimson LIMS [24] was used for retrieval of clinically ordered VRE rectal surveillance swabs and *C. difficile*–positive stool samples over the 246-day study period. Clinical VRE-swab surveillance is performed only in the hospital ICUs. Swab retrieval occurred from days 48 to 199 from medical, surgical, and neurological ICUs (Figure 1), and *C. difficile* toxin B–positive stool collection throughout the hospital over the entire study period. Stool toxin B was detected by enzyme immunosorbent assay (EIA; C. diff Quik Chek Complete; Abbott, Abbott Park, IL), Samples with indeterminate results were tested by polymerase chain reaction (PCR) (GeneXpert *C. difficile*; Cepheid, Sunnyvale, CA). Patients with suspected or confirmed *C. difficile* infection are isolated under contact precautions, following Infectious Diseases Society of America guidelines [23], with donning of gowns and gloves by personnel upon room entry. Patient demographic and contact data were retrieved from the Partners Research Patient Data Registry and Theradoc [25], and were de-identified for analyses (Table 1, Supplementary Table 1).

Validation of VRE Swabs for Detection of *Clostridioides difficile* Carriage

Control *C. difficile* isolates from prior patient samples were grown in brain-heart infusion broth and serially diluted from 1×10^5 to 10^2 colony-forming units (CFU)/mL. To follow clinical workflows, BD BBL CultureSwabs (BD, San Jose, CA) were inoculated with 100 µl of dilution, placed in transport tubes, and stored aerobically for 2 hours, then streaked to Spectra VRE agar (Thermo Scientific, Waltham, MA), returned to their transport tubes, and stored for another 2 hours prior to streaking to CHROMID *C. difficile* agar (Biomerieux, Durham, NC) with anaerobic incubation at 37°C for 24 hours. The input and recovered CFUs were quantified to assess sensitivity of detection and recovery (Figure 2).

Sample Collection

Brigham and Women's Hospital ICU patients are screened for VRE by rectal swab upon ICU admission and weekly thereafter. *C. difficile* toxin B–positive stool samples were retrieved after clinical testing. Samples were plated onto CHROMID *C. difficile* agar. *Clostridioides difficile* colonies were identified as gray to black colonies on CHROMID agar and were speciated by rapid ANA panels (Biomerieux, Durham, NC).

Genomic Analyses

Genome sequencing by Illumina MiSeq was performed as described (Illumina, San Diego, CA) [24]. Genome assembly was done using SPAdes [26] (Supplementary Data File 1). Toxin typing used reference *tcdA* and *tcdB* toxin genes from CD630 (AM180355.1) and the *cdt* toxin gene from strain R20291 (NC_013316.1). Gene calling used cutoffs of 80% reference gene length and 80% amino acid sequence identity. Single nucleotide polymorphism (SNP)–based analyses used the NCBI Pathogen Detection Isolates Browser (https://www.ncbi.nlm.nih.gov/pathogens/isolates) [22].

Clostridioides difficile Phylogenetic Analyses

Clostridioides difficile genomes from the Sequence Reads Archive (SRA) (https://ncbi.nlm.nih.gov/SRA), and NCBI Pathogen Detection Isolates Browser were downloaded for analyses. SPAdes draft assembled genomes passing the following

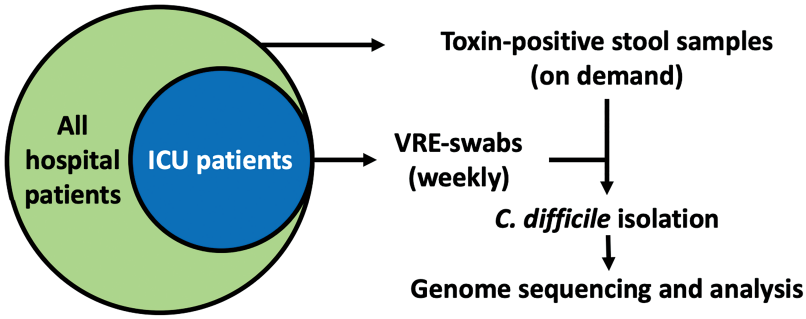

Figure 1. Patient group and sample collections. ICU patients are a subset of all hospital patients. *Clostridioides difficile* isolation and strain sequencing were performed on toxin-positive stool samples from all hospital patients and on all VRE swabs collected from ICU patients. Abbreviations: ICU, intensive care unit; VRE, vancomycin-resistant enterococci.

Table 1. Patient Demographic Data by *Clostridioides difficile* Colonization and *Clostridioides difficile* Infection Status

Parameter	ICU Patients			Non-ICU Patients	
	Noncolonized	Asympto-matically Colonized	CDI	CDI	*P*
Number of patients	1734	135	28	150	
Mean (SD) age, years	62.5 (16.0)	62.0 (15.7)	57.9 (15.9)	60.7 (15.3)	.317[a]
Sex, % female	42.3	43.2	37.0	51.9	.179[b]
Mean (SD) inpatient days across admissions	13.8 (13.9)	26.2 (12.1)	38.8 (27.7)	14.1 (16.3)	<.001[a]
Mean (SD) no. of inpatient admissions	1.33 (0.8)	1.61 (1.05)	2.04 (1.12)	1.38 (1.48)	<.001[a]
Mean (SD) inpatient admission length of stay, days	11.9 (16.4)	18.9 (15.9)	26.3 (22.8)	13.8 (11.8)	<.001[a]
Mean (SD) outpatient hospital visits	5.03 (7.39)	4.79 (7.34)	5.36 (9.14)	5.33 (7.45)	.013[a]
% Mortality within 30 days of last swab or stool culture	17.3	28.9	17.9	16.7	.009[b]

Abbreviations: CDI, *Clostridioides difficile* infection; ICU, intensive care unit; ICU Patients, patients who spent time in an ICU over the study period; Non-ICU Patients, patients not in an ICU over the study period but who had a stool *C. difficile* isolate.

[a]Hypothesis testing by Kruskall-Wallis test.

[b]Hypothesis testing by chi-square analysis.

criteria were used: genome sequence length within 3.7–5.0 Mb and <150 contigs, an L90 <30 (fewest number of contigs covering 90% of assembly), and an average coverage >25X. Analyses evaluated 3377 genomes in 173 SNP groups (Supplementary Data File 2).

A core *C. difficile* genome was created using tblastn with the CD630 reference genome. Feature identification used cutoffs of 80% or higher protein sequence identity and feature length within 20% of the reference protein length. Nucleotide alignments of the extracted genes used MAFFT [27].

A phylogeny of NCBI Pathogen Detection Isolates Browser isolates used up to 3 members from each SNP group. A 95% core genome of 2835 genes resulted in a 422 008b SNP matrix. Of these SNPs, 353 060 occurred in at least 95% of aligned positions, which was used to calculate a phylogeny using RAxML with 1000 bootstraps and GTRCAT [28]. The clade structure concurred with previous analyses [29] and includes 157 SNP clusters, 256 nonclustered single isolates, and several paraphyletic sequence types [30]. The tree is available online at https://itol.embl.de/tree/1702232072546 5491568233605.

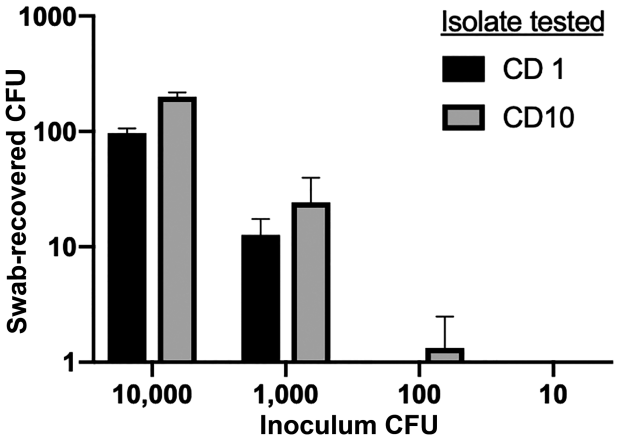

Figure 2. Sensitivity of VRE-swabs for *Clostridioides difficile* retrieval by culture. The *x*-axis shows the *C. difficile* CFU in inocula applied to VRE swabs before plating to VRE media, per standard clinical protocol and, 2 hours later, to CHROMID *C. difficile* agar. The *y*-axis shows recovered *C. difficile* CFU. Abbreviations: CFU, colony-forming units; VRE, vancomycin-resistant enterococci.

Statistical Analyses

Statistical analyses used the Python package SciPy [31]. Differences in demographic data between patient groups were calculated using the Kruskal-Wallis test and Mann-Whitney post hoc test for continuous variables and χ^2 test for discrete variables. Multi-hypothesis–adjusted *P* values were calculated using the Benjamini Hochberg procedure [32]. Relative risk ratios were calculated by dividing the probability of developing CDI in the asymptomatically colonized (exposed) group of patients by the probability of developing CDI in the control (noncolonized) group (Table 2).

RESULTS

Clostridioides difficile Surveillance Program

The CDI genomic screening pilot evaluated 2432 VRE swabs from 1897 ICU patients over 152 days, from which 172 *C. difficile* isolates (7.1% of swabs) were identified in 143 patients (7.5% of ICU patients) (Figure 1, Table 1, Supplementary Table 1). Asymptomatic colonization with *C. difficile* occurred in 7.4% of ICU patients (n = 140), including 5 who later developed CDI as diagnosed by toxin B stool testing. An additional 3 patients had CDI prior to culture of *C. difficile* from swabs. A total of 28 ICU patients (1.5% of ICU patients) had CDI diagnosed over the study period, 20 of whom did not have a prior positive swab.

Hospital-wide, toxin B–positive stool samples from ICU and non-ICU patients were cultured for *C. difficile* to define the genomic diversity of strains causing infections. A total of 178 toxin B–positive stool samples were cultured, with 98.3% of stool samples (n = 174) growing a *C. difficile* isolate. Of these, 16.1% (28 isolates of 174) were from ICU patients.

Table 2. Relative Risk of *Clostridioides difficile* Infection per Toxin Status of Carried *C. difficile* Isolates in Intensive Care Unit Patients

Relative Risk From Carriage of Toxin-encoding Strains	Developed CDI	Did Not Develop CDI
ICU patients carrying a toxin-encoding strain	5	89
ICU noncarriers and non–toxin-encoding carriers	10	1742
Relative risk from carriage of non–toxin encoding strains	Developed CDI	Did not develop CDI
ICU patients carrying a non–toxin-encoding strain	0	45
ICU noncarriers	15	1786

CDI relative risk from carriage of toxin-encoding *C. difficile*: 9.32; 95% confidence interval: 3.25–26.7; $P < .001$. CDI relative risk from carriage of non–toxin-encoding *C. difficile:* 1.26; 95% confidence interval: .08–20.8; $P = .87$.

Abbreviations: CDI, *Clostridioides difficile* infection; ICU, intensive care unit.

Association of Colonization Status With Hospital Admissions and Mortality

The ICU patients with asymptomatic *C. difficile* colonization or infection had an association with increased inpatient days across all admissions during the study period, as compared with noncolonized ICU patients (Table 1, row 4). ICU patients who developed CDI during the study period averaged 38.8 inpatient days and asymptomatically colonized ICU patients 26.2 days versus 13.8 days for noncolonized ICU patients ($P < .001$).

Asymptomatic carriage or CDI in ICU patients was also associated with increased lengths-of-stay per hospital admission, defined as days from admission to release (Table 1, row 5). ICU patients diagnosed with CDI demonstrated an average of 26.3 days per admission versus 18.9 for ICU asymptomatic carriers and 11.9 days for noncolonized ICU patients ($P < .001$).

Asymptomatically colonized versus noncolonized ICU patients also showed differences in the number of repeat admissions over the study period at 1.61 versus 1.33 admissions ($P < .001$) (Table 1, row 6). In contrast, ICU patients who developed CDI had higher repeat admissions of 2.04 ($P = .017$). Mortality rates by 30 days after the end of the rectal swab collection period were also higher in asymptomatically colonized versus noncolonized ICU patients at 28.9% versus 17.3%, respectively ($P = .006$) (Table 1, row 8).

Genomic Diversity of Hospital *Clostridioides difficile* Isolates

Strain genomic data were submitted to the NCBI Pathogen Detection resource to evaluate *C. difficile* hospital SNP clusters relative to datasets from other centers. Clusters were analyzed for clade designation and sequence type (ST). The 346 isolates from 309 patients were highly diverse and occurred primarily in clades 1 and 2 (Figure 3A and 3B). Within clade 2, 20 isolates clustered to ST 1 (NAP1/RT027, *cdt* toxin positive; 5.8% of isolates). Clade 2 strains were more than twice as likely to originate from toxin B–positive stool samples (24 isolates) than from VRE screening swabs (11 isolates; $P = .035$). The *cdt* toxin locus was identified in strains from clade 5, primarily in ST11 from SNP cluster PDS000017348 [33]. The CDT-encoding strains occurred more frequently from stool (n = 30) than VRE swabs (Figure 3C and 3D) (n = 16; $P = .044$).

Clade 4 included 2 distinct genetic groups, including ST37 isolates encoding toxin B but not toxin A, of which 7 originated from stool samples and 1 from a VRE swab, a proportion differing significantly from the remaining non–toxin-encoding clade 4 strains (n = 5; $P = .01$).

Among asymptomactially colonized ICU patients, 31.8% of isolates were non–toxin-encoding lacking both the *tcd* and *cdt* loci (53 isolates from 45 patients).

Longitudinal Strain Dynamics in Asymptomatic Intensive Care Unit Carriers

Twenty-five ICU patients (15.3% of all swab-positive patients), including 17 asymptomatic carriers, demonstrated longitudinal *C. difficile* carriage (Figure 4A). Within these isolate genomes, the maximum number of SNPs was 17, with an average of 7.5 SNPs per asymptomatic patient.

Clostridioides difficile Infection Occurrence in Asymptomatic Carriers

Five asymptomatic ICU carriers developed active CDI (Figure 4B), representing 3.5% (5 of 140) of all asymptomatic carriers and 18% (5 of 28) of ICU patients who developed CDI. In 4 cases, the VRE-swab isolate was within 5 SNPs of the stool isolate and was considered clonally related. The fifth case, patient 57, developed CDI with a different epidemic ST37 strain, which occurred 3 days after detection of asymptomatic carriage. Four of five CDI diagnoses occurred within 1 week of asymptomatic carriage detection, while patient 45 was diagnosed 91 days after detection. Five separate ICU patients also had *C. difficile*–positive VRE swabs after CDI diagnosis. In patients 69 and 171, the swab isolates were distinct from the stool isolate, suggesting potential acquisition after clearance from treatment.

Relative Risks for *Clostridioides difficile* Infection From Asymptomatic Carriage of Toxin-encoding *C. difficile*

The relative risk for developing CDI from asymptomatic carriage of a toxin-encoding strain was 9.32 (95% confidence interval [CI], 3.25–26.7; $P < .001$). The 5 CDI cases from 140 asymptomatic ICU patients represented 3.6% of asymptomatic carriers. In contrast, carriage of a non–toxin-encoding strain did not increase risks for CDI (relative risk, 1.26; 95% CI, .08–20.8, $P = .87$); no carriers of non–toxin-encoding strains developed CDI over the study period (Table 2).

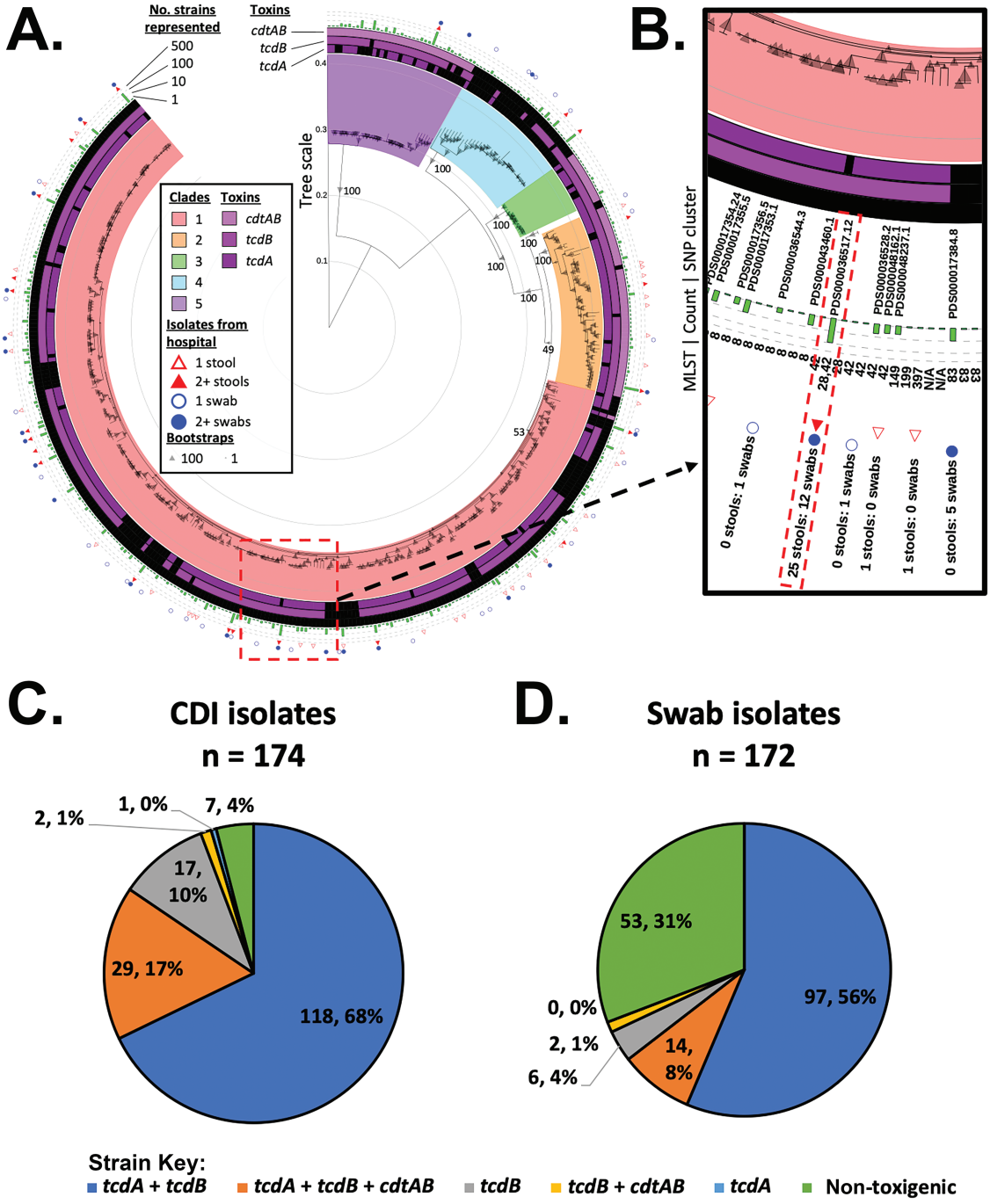

Figure 3. BWH patient isolates are genomically diverse. *A*, *Clostridioides difficile* SNP cluster tree of 157 SNP clusters, represented by 1 isolate, and 256 nonclustered isolates are shown. The outer ring shows the source of strains present in the branch (stool or VRE swab) and number of isolates. A bar graph in the second ring indicates the number of strains the branch represents in the NCBI Pathogen Detection Isolates Browser. The inner rings indicate presence (purple) or absence (black) of different toxin genes found within each clade. Clades are indicated by a colored box around the interior nodes that compose the clade. *B*, Inset of panel *A* showing an interactive view available online with the sequence types and strains represented in each leaf. *C* and *D*, Percentage of different toxin types from stool (*C*) or VRE swabs (*D*). The difference in non–toxin-encoding strains found in swabs versus stool samples is significant at $P < .001$. Abbreviations: BWH, Brigham and Women's Hospital; CDI, *Clostridioides difficile* infection; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism; VRE, vancomycin-resistant enterococci.

Genomic-epidemiological Investigations of Asymptomatic Transmission

Genomic cluster analyses identified related subclusters of isolates for spatial-temporal analyses to assess potential transmission events (Figure 4). Per the relatedness of longitudinal isolates from the same patient, thresholds of fewer than 17 SNPs and individual branch lengths within clusters of fewer than 15 SNPs were used to define clusters for analyses. Analyses identified 28 subclusters across 20 SNP groups, involving 76 isolates

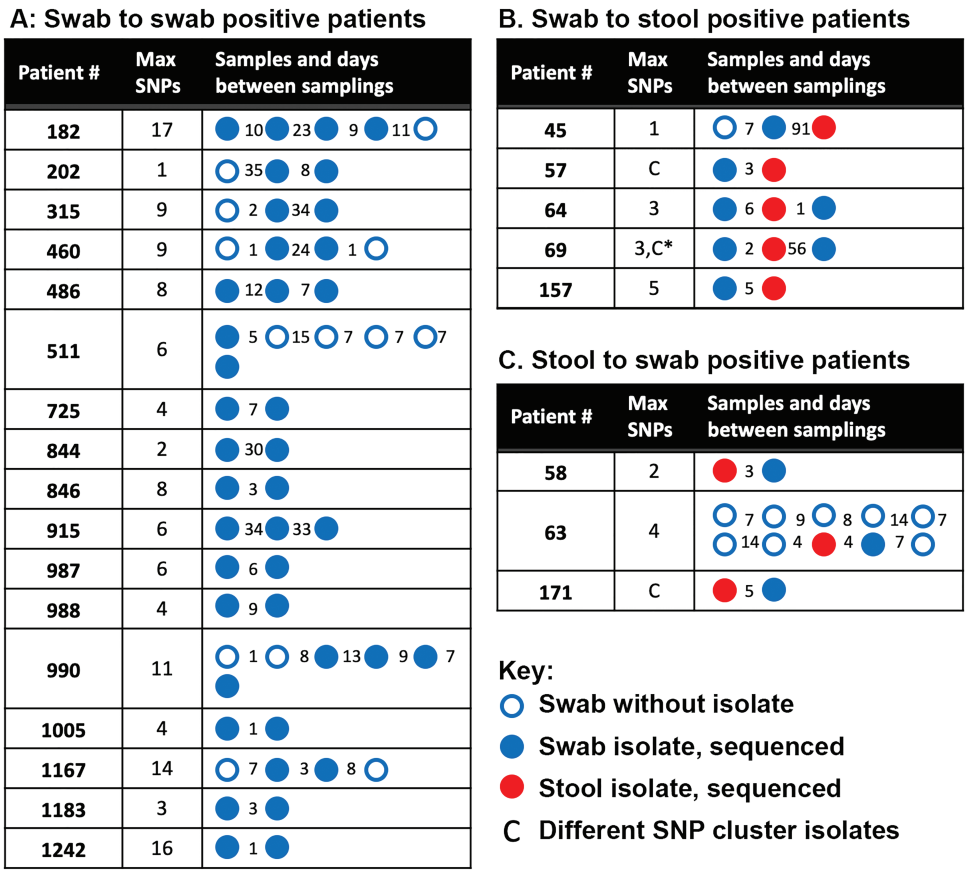

Figure 4. Longitudinal *Clostridioides difficile* carriage. Patient samples are indicated with circles. Blue indicates a VRE swab and red a toxin-positive stool sample. Filled circles are samples that produced isolates for sequencing. The number of days between samples is indicated by the number between the circles. *A*, Asymptomatic carriers. Carried isolates remained in the same SNP cluster for all patients. *B*, Asymptomatic carriers who developed CDI. In patient 57, a strain from a different cluster was cultured from the positive stool sample. In patient 69, a strain different from the previously carried and CDI-causing strain was detected 56 days after CDI diagnosis and treatment. *C*, Patients with CDI with subsequent asymptomatic carriage. In patient 171, a different strain was identified 5 days after CDI diagnosis and start of therapy. Abbreviations: CDI, *Clostridioides difficile* infection; SNP, single nucleotide polymorphism; VRE, vancomycin-resistant enterococci.

from 65 patients (Supplementary Data File 3). Analyses evaluated spatial-temporal linkages among hospital floors, wings, rooms, and bed spaces.

Within the 28 clusters, the largest group of genomically related strains occurred in SNP cluster PDS000036517 (Figure 5A), which included 19 isolates from the present study, 12 prior isolates from BWH, and 4 from the University of Pittsburgh (Figure 5B). Isolates within this subcluster were 2–34 SNPs apart, ruling out a single outbreak cluster. Three groups of BWH isolates within this subcluster met criteria for investigation but did not show subsequent spatial-temporal links among patients.

However, spatial-temporal overlaps among patients were identified in 13 other clonal clusters. These overlaps occurred from 2 to 210 days prior to culture of *C. difficile* isolates, with a median of 37 days (Figure 6, Supplementary Figure 1). Among cases, 26 of 28 patients shared a floor location, 15 shared a wing location, 3 a room, and 2 a bed space. Using a Poisson cumulative probability cutoff of 0.1, an investigative threshold used by local hospital-infection control teams to flag clusters for evaluation, the period where a repeat observation on the same floor could be considered linked is 258 days, and 447 days for observations from the same wing. The 28 cases flagged by initial genomic analyses of related strains fell within these time constraints.

Figure 6A shows an example analysis for asymptomatic transmission in genomically identified strain clusters. Patient 460 produced swab isolate V687 on day 105, 2 days after ICU admission. The patient remained colonized 24 days later during a second hospital admission to a different floor. Patients 949 and 700 had contact with the same ICU bed space and ICU wing, respectively, several weeks after patient 460's discharge. Patient 949 was confirmed to be noncolonized by VRE swab upon ICU admission. Both patients tested positive for *C. difficile* by VRE swab after this exposure. Patients 460 and 949 both tested negative for *C. difficile* by stool EIA. While these patients were showing symptoms that led to CDI testing, the negative toxin EIA results suggest that the biomass of colonizing *C. difficile* was not elaborating sufficient toxin for detection. The 3 cases

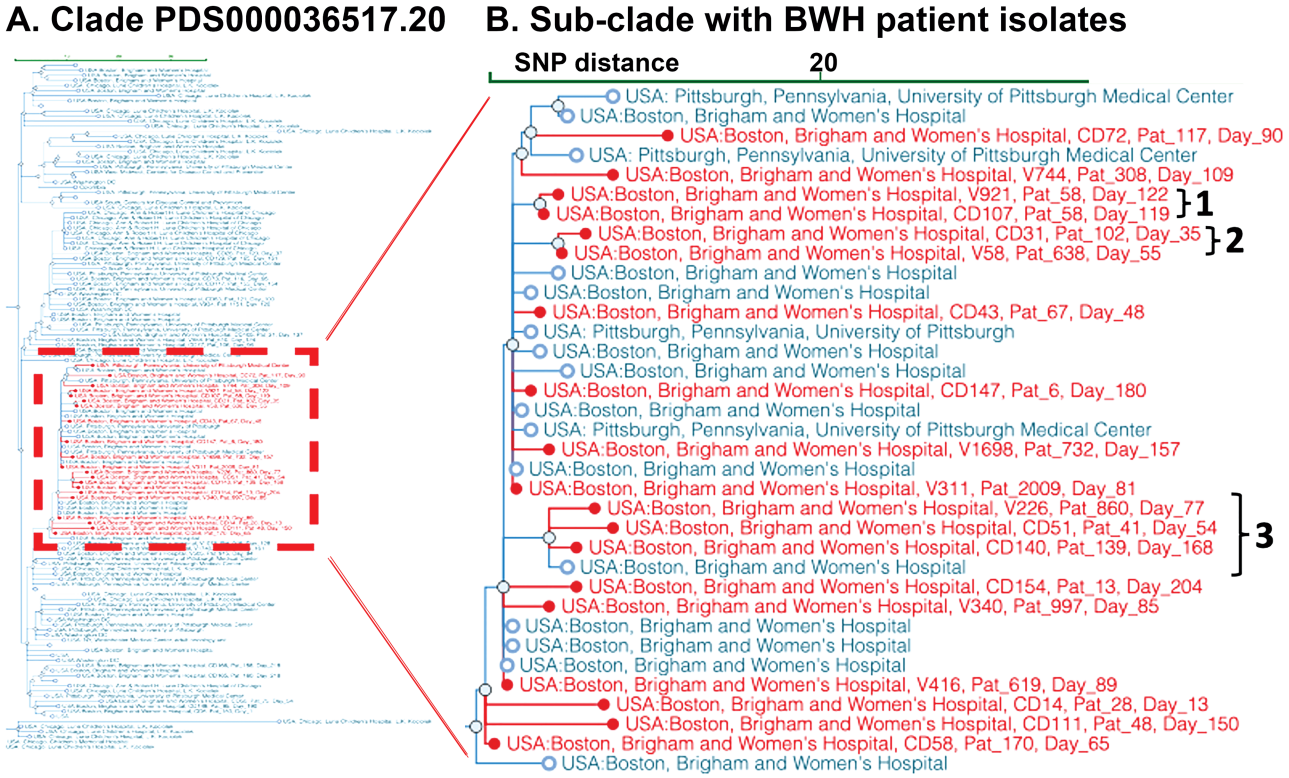

Figure 5. Subclade selection of *Clostridioides difficile* genomic clusters by institution and region. The NCBI Pathogen Detection Isolates Browser provides a comparison tool for identifying outbreaks and relating them to other submitted isolate genomes. *A*, Subclade PDS000036517.20 (135 isolates). *B*, Subcluster of 35 strains within this clade that includes the largest set of related isolates (n = 19) from the present study (red). Blue entries show 16 prior samples from BWH and 4 from the University of Pittsburgh. The de-identified patient identifier and study day of isolate isolation are overlaid on the NCBI tree. "1" Shows 2 isolates from the same patient; "2" shows 2 closely related isolates that occurred within 20 days but with no identifiable patient spatial overlaps; "3" shows 5 isolates forming a related subclade that includes a sample from BWH submitted 2 years prior to the study (blue). Abbreviations: BWH, Brigham and Women's Hospital; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism.

thus represent potential asymptomatic transmission events from an initial asymptomatic carrier.

Other potential asymptomatic transmission cases demonstrated longer periods between spatial overlaps, including up to 52 days apart (Figure 6B–F, Supplementary Figure 1).

DISCUSSION

This study provides the first detailed genomic and epidemiologic analyses of asymptomatic *C. difficile* carriage in ICU patients. The relative risk for developing active infection from asymptomatic carriage of a toxin-encoding strain was 9.32. Carriage was also associated with increased hospital lengths of stay and re-admissions during the study period, as well as 67% increased mortality in carriers when compared with mortality rates in noncolonized ICU patients. However, these findings are not definitively causal relative to other clinical factors, including that asymptomatic carriers had higher overall exposure to the healthcare system, a factor that increases risks for *C. difficile* colonization. The potential for asymptomatic carriage to cause subclinical disease in patients with underlying comorbidities nonetheless raises a critical question on benefits of ICU screening for *C. difficile* to identify carriers, not only to reduce reservoirs for transmission but to also reduce longer-term comorbidities and mortality in carriers. Screening of vulnerable patient populations for *C. difficile* carriage also has the potential to inform use of antibiotics and other clinical interventions to reduce risks for CDI [34].

Integrated genomic and epidemiologic analyses identified multiple potential transmission events from patients with CDI and asymptomatic carriers to other patients. Analyses identified a 258-day window in which patient spatial overlaps were significantly associated with potential transmission.

Asymptomatic carriers longitudinally carried the same strain, sometimes over months [35, 36]. Furthermore, asymptomatically carried strains caused active CDI in 4 of the 5 cases identified. In cases of extended periods between identification of asymptomatic carriage and CDI, we note that our findings cannot rule out infection from direct carriage versus strain re-introduction from spores that persist in the patient's environment [37].

We validated a culture-based method for *C. difficile* screening, leveraging rectal swab samples from an existing

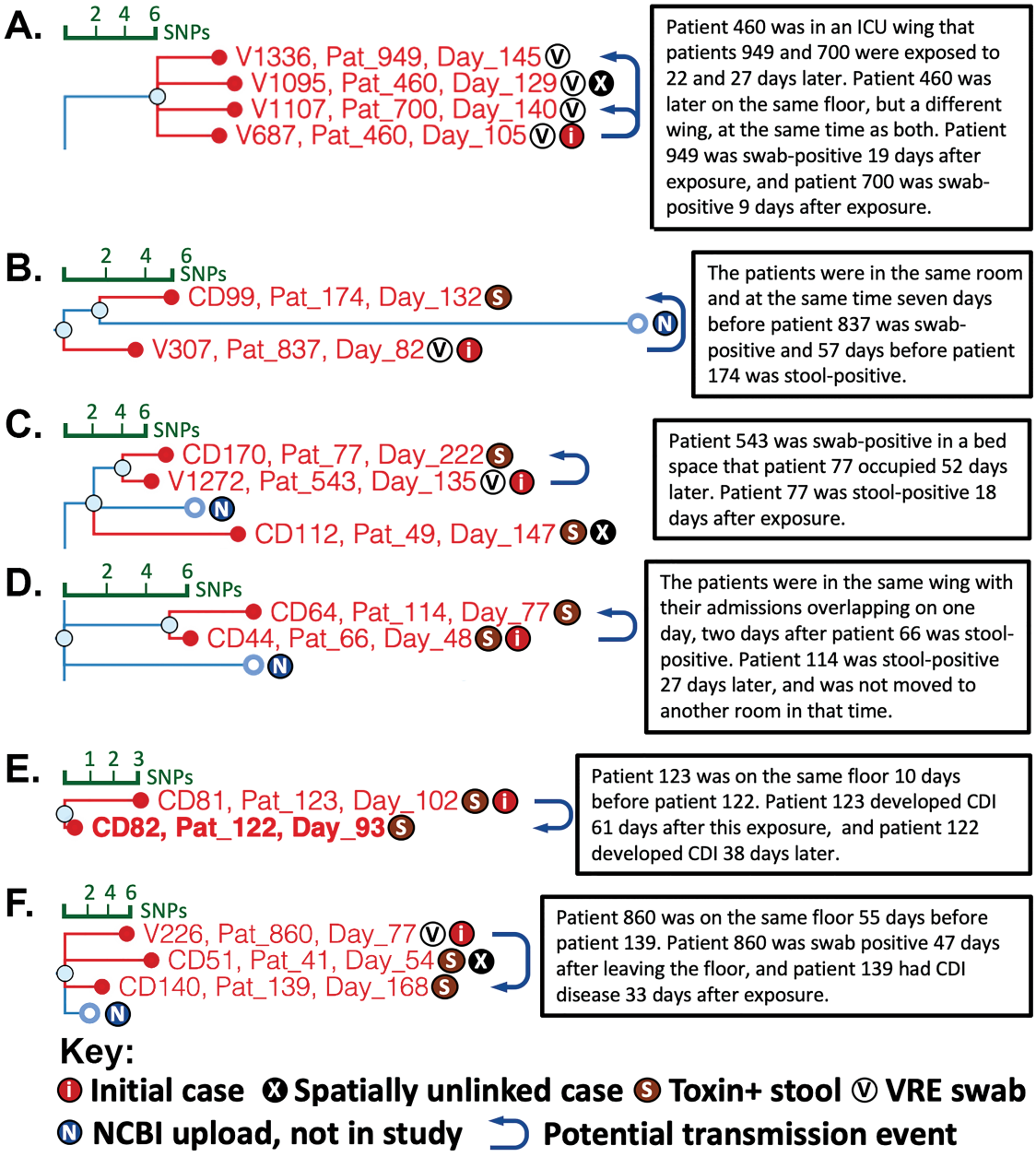

Figure 6. Cases with genomic and spatial-temporal evidence for nosocomial transmission. SNP tree branches from the NCBI Pathogen Detection Isolates Browser are overlaid with the isolate identifier, de-identified patient number, and day within the study that the sample was collected. The key at the bottom indicates cases and sample types from which *Clostridioides difficile* was isolated. Arrows indicate potential transmission events based on genomic and hospital epidemiologic analyses of patient hospital location data. *A–F*, Example cases. Text boxes summarize supporting spatial and temporal information from integrated genomic-epidemiologic analyses. Abbreviations: CDI, *Clostridioides difficile* infection; ICU, intensive care unit; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism; VRE, vancomycin-resistant enterococci.

VRE surveillance program to reduce the complexity of implementation. For centers with anaerobic culturing capabilities, screening costs include the selective agar, species and toxin confirmation, quality programs for testing, and efforts of clinical laboratory and infection-control personnel to perform the testing and act upon results. The majority of cultures are negative and can be reported within 24 hours, offering a more cost-effective screening option over molecular methods [13, 20, 21]. As 30% of asymptomatic carriers were colonized with non–toxin-encoding strains, a finding that did not elevate risks for CDI, confirmation of toxin production or carriage by EIA or PCR is warranted. Swab-based detection of toxin-encoding *C. difficile* preceded 18% of ICU CDI cases, providing an opportunity for early interventions, while also potentially preventing further asymptomatic transmission. Hospital-onset CDI costs an average of more than $34 000 per patient. Thus,

prevention of even a subset of cases can bring significant savings [38].

A study placing *C. difficile*–colonized patients on contact precautions saw significant reductions in CDI [15]. As 5.1% of ICU-admitted patients were found to carry a toxin-encoding strain of *C. difficile*, the number of patients put on contact precautions would increase, potentially introducing burdens on clinical infrastructure. Informed by local rates of *C. difficile* carriage and CDI, healthcare facilities can assess the utility of screening by incorporating logistical and economic costs, as well as clinical actions to take upon identifying asymptomatic carriage [39, 40].

Our epidemiologic analyses used an SNP cutoff of up to 17 SNPs to flag potential clonal clusters, a cutoff defined from analyses of longitudinal isolates from the same patient. Incorporation of strain genomic and patient spatial-temporal information identified potential *C. difficile* transmission events among 65 patients, 21% of the 315 patients who produced isolates during the study. Analyses also validated the use of publicly available SNP calling tools for *C. difficile* in the NCBI Pathogen Detection Isolates Browser. As more institutions contribute *C. difficile* genomic data, higher resolution analyses may be undertaken, particularly given the widespread nature of CDI across healthcare institutions.

Asymptomatic carriers of *C. difficile* provide a significant and hidden pathogen reservoir that can have adverse effects for carriers, other patients, and healthcare workers. We demonstrate constructive use of existing hospital surveillance programs and nationally available genomic tools and resources for *C. difficile* surveillance within an ICU setting. Leveraging this model, institutions can make informed decisions regarding the utility of screening to reduce CDI incidence.

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.

Notes

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