# Longitudinal genomic investigation of *Clostridiodes difficile* transmission in a densely sampled ICU cohort

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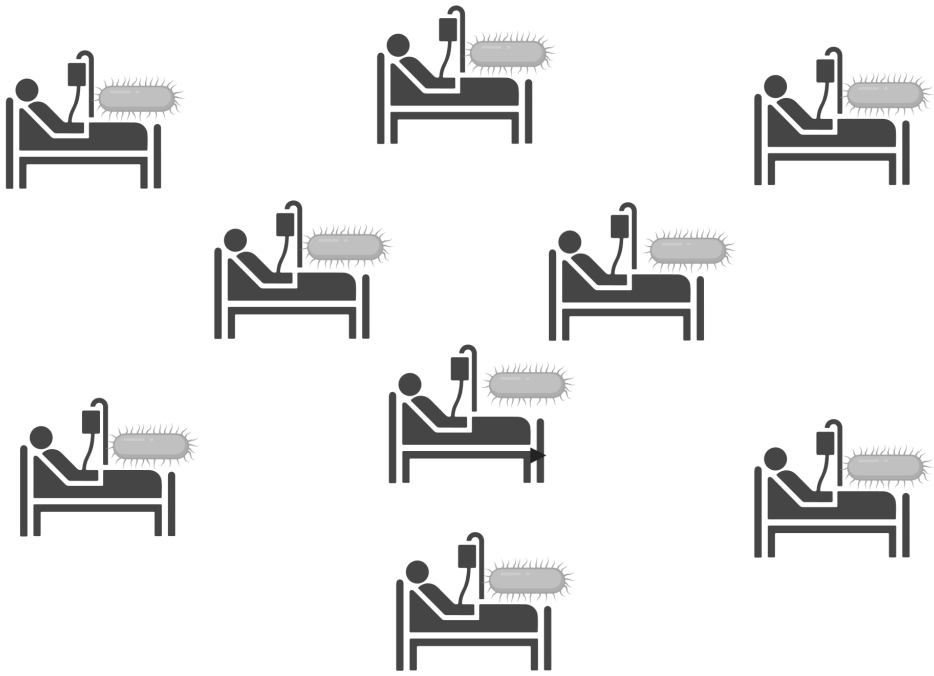
# Genomic epidemiology to study healthcare-associated pathogens

- Whole-genome sequencing (WGS) has revolutionized our ability to track the spread of pathogens
- The unprecedented resolution of WGS enables insights into whether two patients harbor pathogens that are plausibly linked by transmission in the facility
- In healthcare settings, WGS is increasingly being used in the context of routine infection prevention



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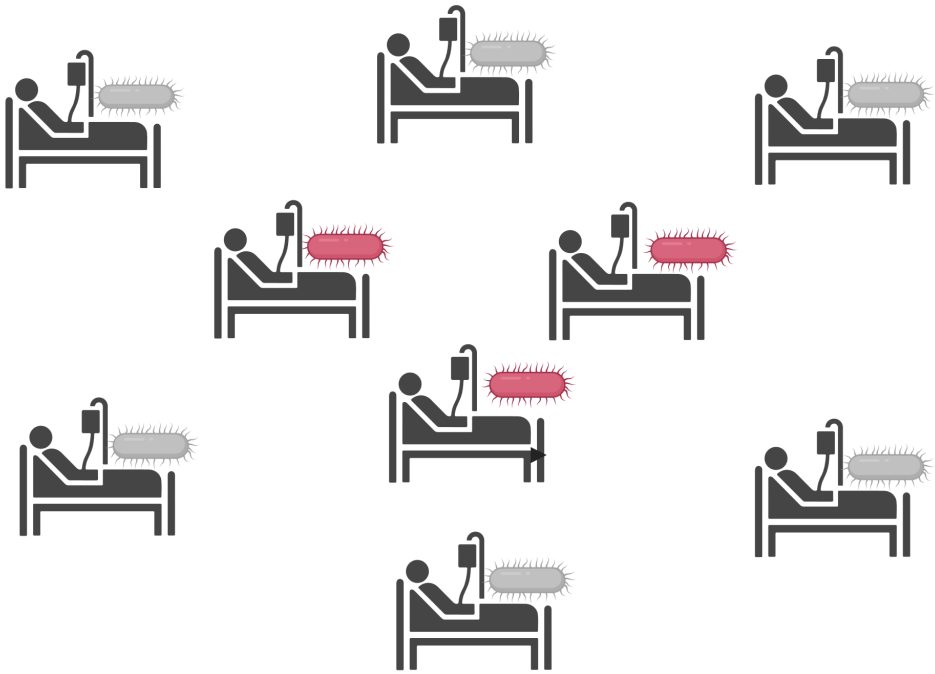

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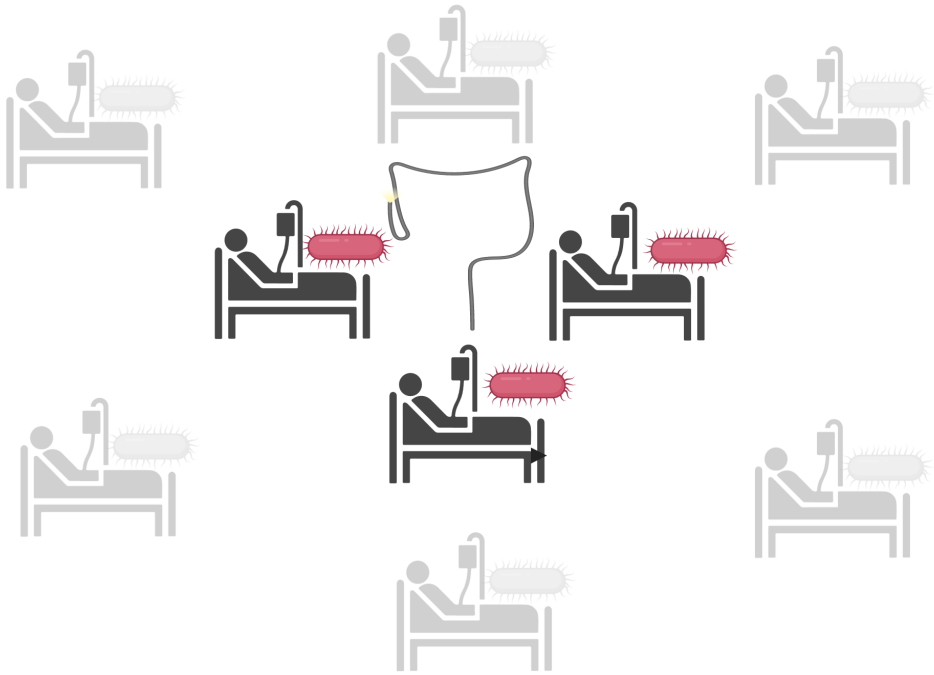
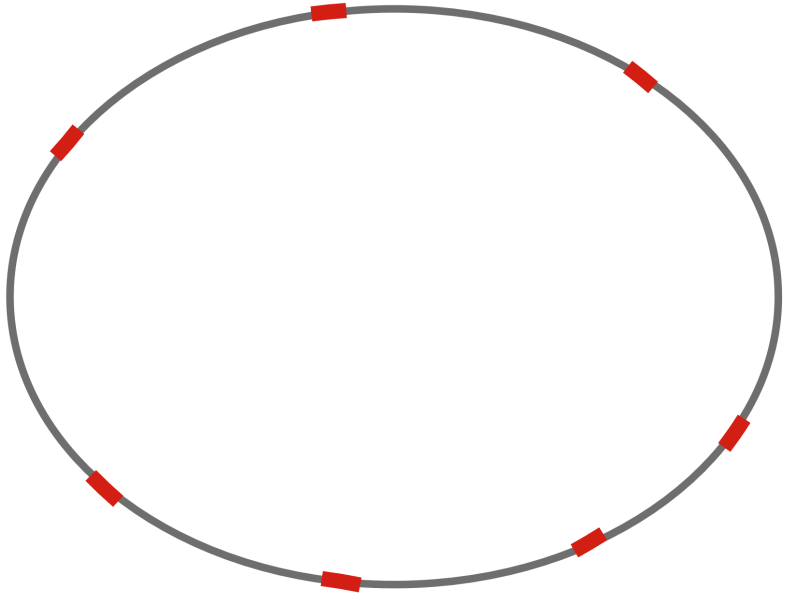


# Some genomic epidemiology jargon

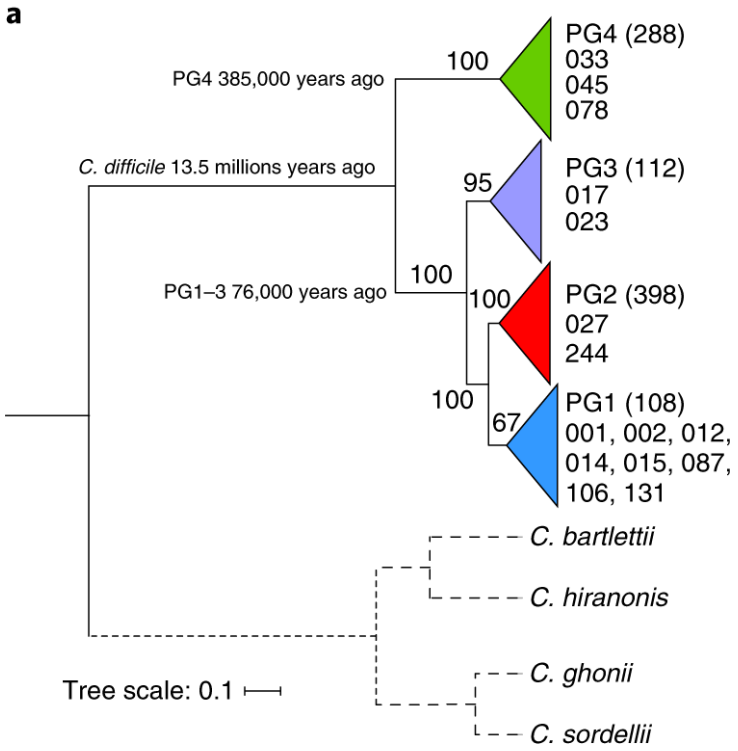
- Sequence type (ST) – a high-level classification of bacterial pathogens based on the sequence at ~7 genes
  - Used to track emerging threats at larger scales
  - Most transmission will be isolates of the same ST

- Single nucleotide variants (SNVs) – Number of positions across the genome where two bacterial isolates vary
  - *C. difficile* has 4 million base pair genome with ~4,000 genes
  - The number of SNVs consistent with transmission varies by bacteria, but is typically less than 20 SNVs



# *Clostridioides difficile* is a genetically and epidemiologically diverse pathogen

- Common ancestor of disease-causing lineages of *C. difficile* date back almost 400K years ago
- Strains can vary by hundreds of thousands of single nucleotide variants, and half their gene content
- *C. difficile* is a mix of toxigenic and non-toxigenic strains, with infection being caused by the toxin producers




Kumar et al., Adaptation of host transmission cycle during *Clostridium difficile* speciation, Nature Genetics 2019

# Strains of *C. difficile* differ in their epidemiology

- Diverse strains underlying burden *C. difficile* infections in the U.S.
- NAP1 (ribotype 027/ST1) and NAP4 (ribotype 014-020/ST2) were historically the dominant strains in the U.S.
- ST1/027 enriched in healthcare facilities, ST2/014 has presence in both hospitals and community

Table 4. Distribution of *C. difficile* Strains, According to Epidemiologic Category.*

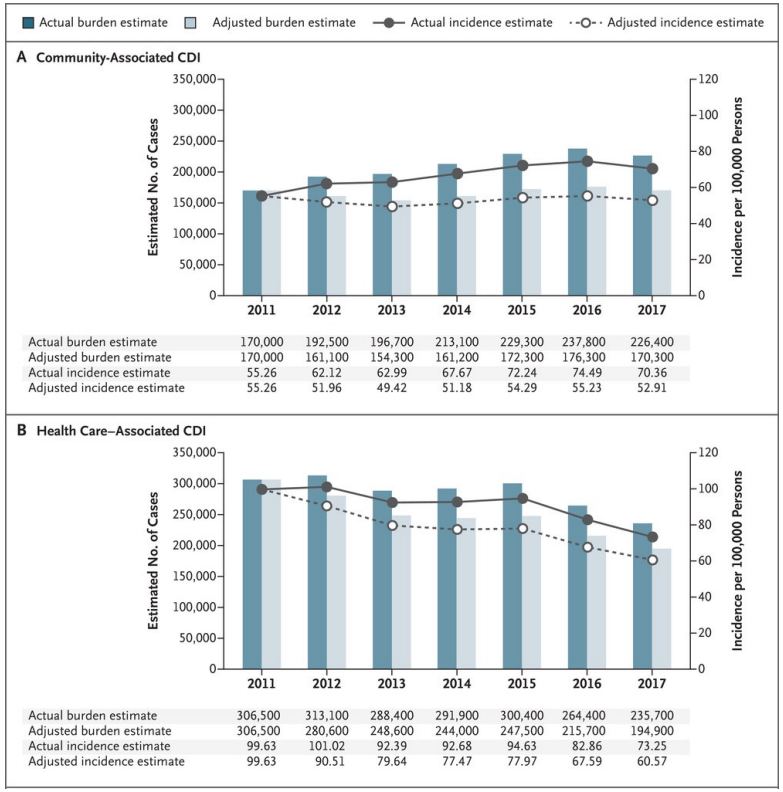
| Strain | Community-Associated CDI (N=735) | Health Care–Associated CDI (N=629) |
|---|---|---|
| | *no. of cases (%)* | |
| NAP1 | 138 (18.8) | 193 (30.7) |
| NAP1-related† | 13 (1.8) | 20 (3.2) |
| NAP2 | 13 (1.8) | 10 (1.6) |
| NAP3 | 3 (0.4) | 12 (1.9) |
| NAP4 | 84 (11.4) | 65 (10.3) |
| NAP5 | 3 (0.4) | 6 (1.0) |
| NAP6 | 56 (7.6) | 27 (4.3) |
| NAP7 | 25 (3.4) | 13 (2.1) |
| NAP7-related‡ | 2 (0.3) | 2 (0.3) |
| NAP8 | 5 (0.7) | 1 (0.2) |
| NAP9 | 22 (3.0) | 9 (1.4) |
| NAP10 | 21 (2.9) | 15 (2.4) |
| NAP11 | 79 (10.7) | 63 (10.0) |
| NAP12 | 9 (1.2) | 16 (2.5) |
| Unnamed§ | 245 (33.3) | 163 (25.9) |
| Could not be typed¶ | 17 (2.3) | 14 (2.2) |

**Lessa, F. C. et al. Burden of Clostridium difficile Infection in the United States. New England Journal of Medicine 372, 825–834 (2015).**

# *Clostridioides difficile* has a presence in both community and healthcare settings

- While often thought of a hospital pathogen, toxigenic *C. difficile* has diverse community reservoirs

- While improvements in infection prevention practices and antibiotic stewardship have decreased health-associated *C. difficile* infection (CDI), community-associated cases have remained flat




Guh et al. **Trends in U.S. Burden of *Clostridioides difficile* Infection and Outcomes**. New England Journal of Medicine, (2020).

How much of the burden of *C. difficile* infection in hospitals stems from healthcare transmission?

# Prior work hints at an under-estimation of hospital-onset CDI stemming from admission colonization

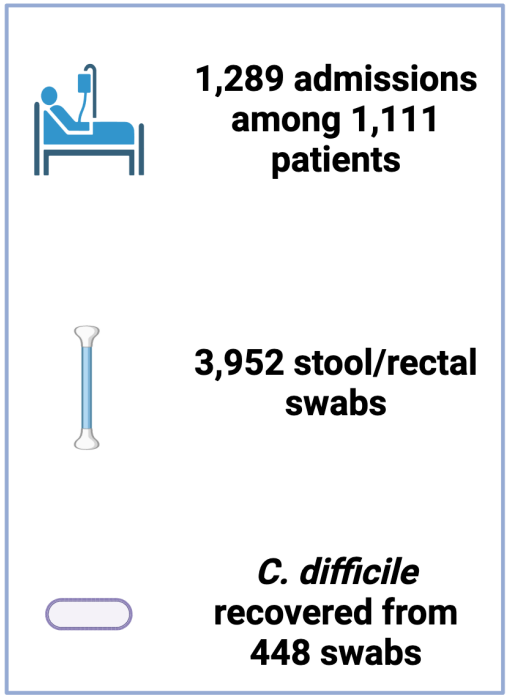
- Seminal work showed that most isolates from CDI cases could not be genetically linked to prior case, indicating acquisition from unsampled reservoirs inside or outside the hospital[1]

- Multiple studies support the risk of developing CDI in the hospital among those with asymptomatic carriage[2]

- However, it's unclear the degree to which asymptomatic carriage is acquired during or prior to hospital admission[3]

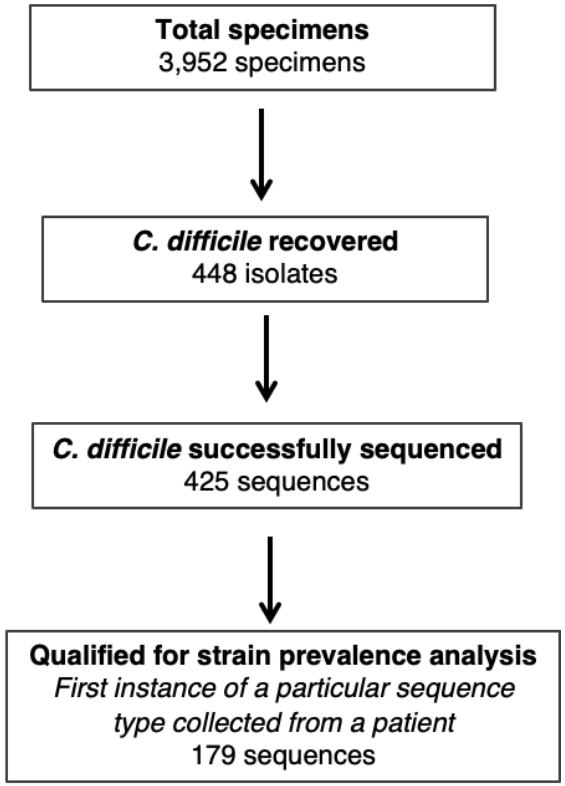

1. Eyre et al., Diverse Sources of *C. difficile* Infection Identified on Whole-Genome Sequencing, NEJM, 2013
2. Zacharioudakis et al., Colonization With Toxinogenic *C. difficile* Upon Hospital Admission, and Risk of Infection: A Systematic Review and Meta-Analysis, AJG, 2015
3. Kong et al., *Clostridium difficile*: Investigating Transmission Patterns Between Infected and Colonized Patients Using Whole Genome Sequencing ,CID, 2019

# Studying transmission and infection using a densely sampled longitudinal cohort




**Daily rectal surveillance for *C. difficile* carriage for all ICU patients over 9-month study**

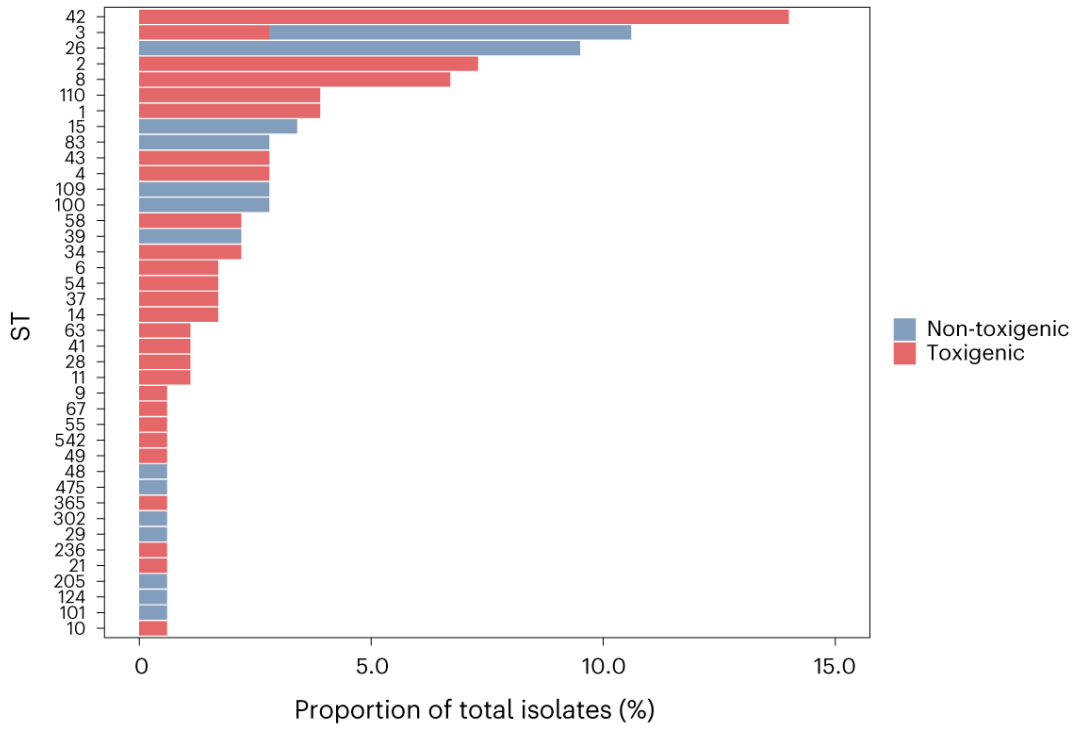
# Overview of *C. difficile* samples recovered and whole-genome sequenced



# Strain dynamics in the ICU over time

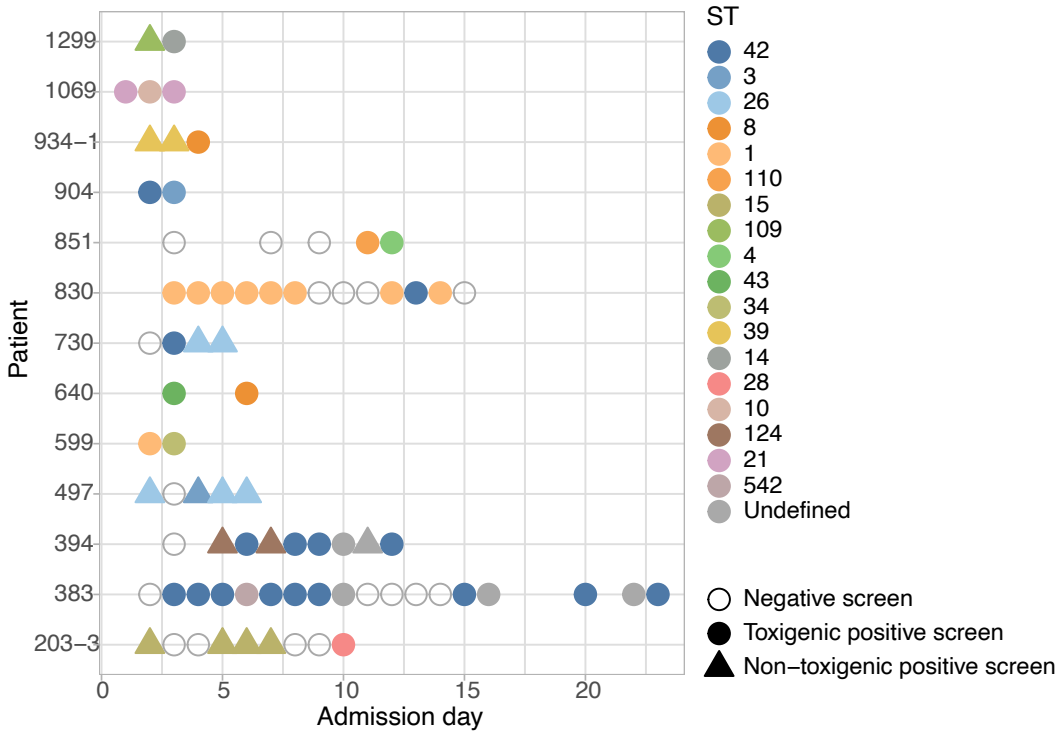
# Overview of 425 *C. difficile* genome sequences isolated from 209 admissions

- All sequence types (STs), except ST3 harbored exclusively toxigenic or non-toxigenic strains
- 40 unique STs detected, with a total of 179 unique patient-ST combinations
- ST1 (ribotype 027) the 7$^{th}$ most prevalent ST, and only detected in 7 patients

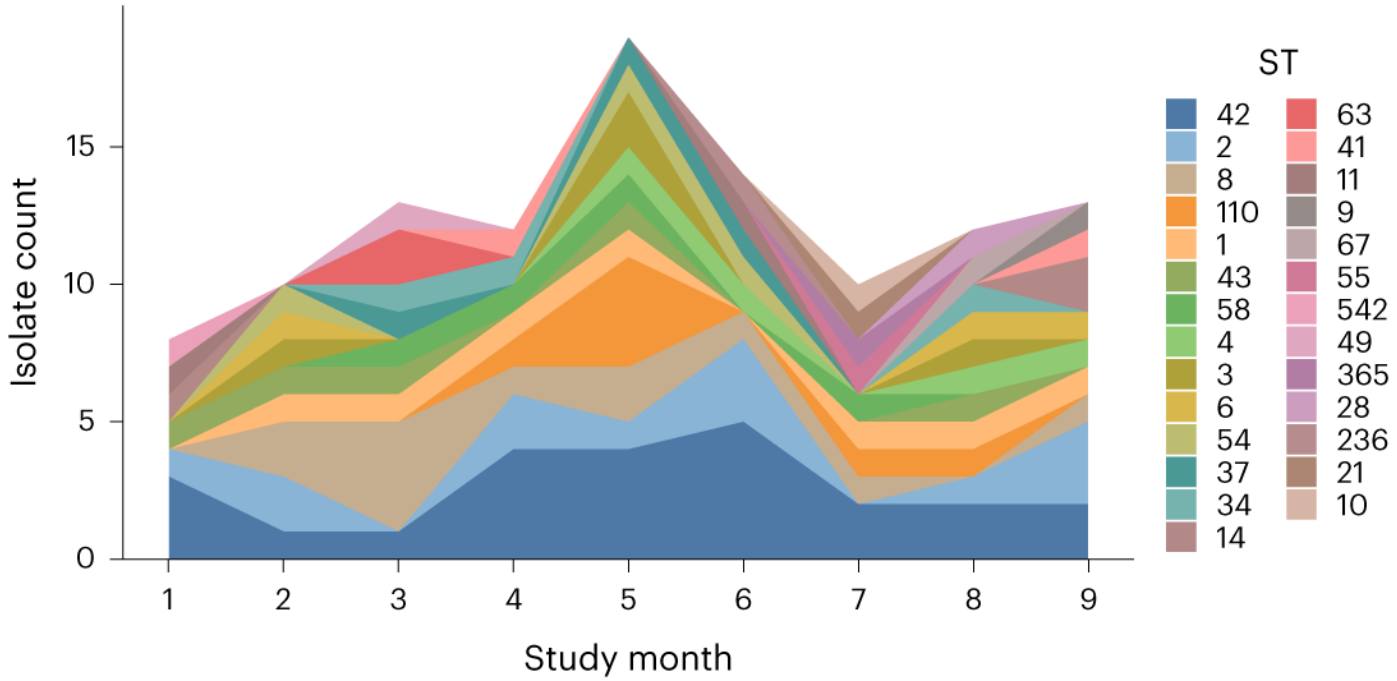


# Patients with multiple STs harbored a mix of toxigenic and non-toxigenic strains

- 13/86 (15%) admissions with multiple isolates had multiple STs detected
- Patients harbored tox/non-tox strains in different combinations (7 tox/tox, 5 tox/non-tox, 1 non-tox/non-tox)



# Common strains of *C. difficile* were present in the unit throughout the study



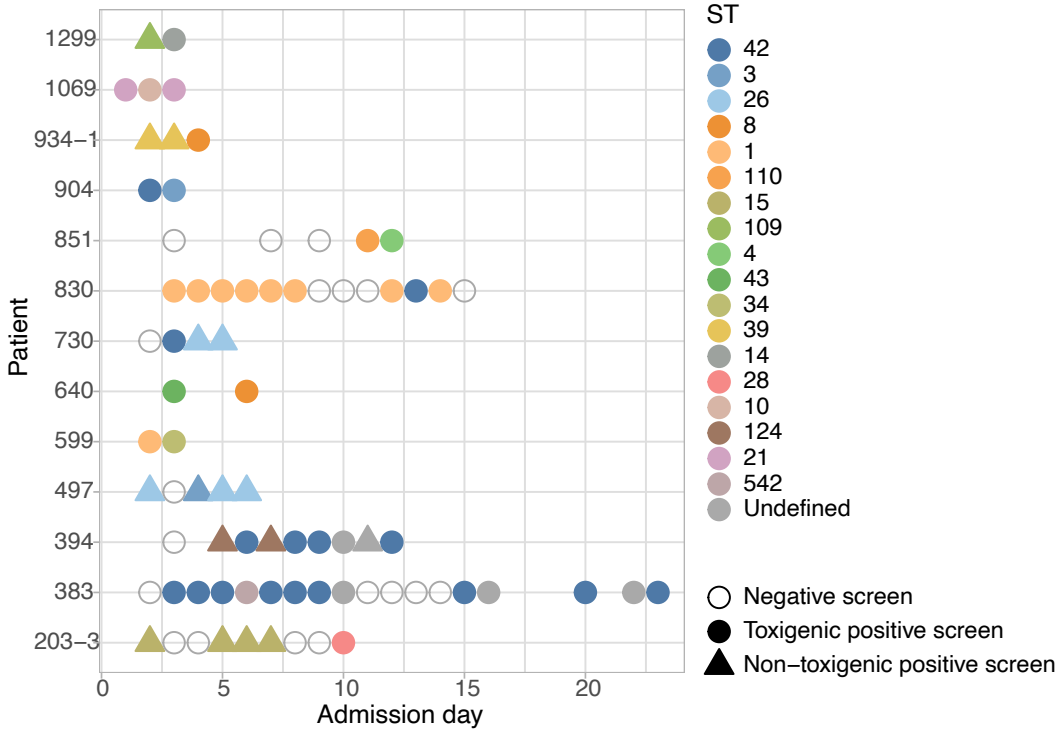
# Transmission dynamics in the ICU

# In total, we detected 120 importation events and 51 culture acquisition events for *C. difficile*

| | Screening prevalence | Importation prevalence | Culture acquisition prevalence | Incidence rate (acquisitions per 100 bed-days) |
|---|---|---|---|---|
| **All *C. difficile*** | 209/1289 (16.3%) | 120/1147 (10.5%) | 51/563 (9%) | 2.1 |
| **Toxigenic only** | 120/1289 (9.3%) | 67/1147 (5.9%) | 32/584 (5.5%) | 1.6 |

# Examining multi-strain colonization does not support protection from non-toxigenic strains

- 584 admissions qualified for acquisition analysis, of which 32 acquired toxigenic *C. difficile*
- 4 toxigenic acquisitions among 27 non-toxigenic importers (14.8%)
- 28 toxigenic acquisitions among 557 individuals with no *C. difficile* detected on admission (5.0%)
- Chi-squared $p < 0.001$



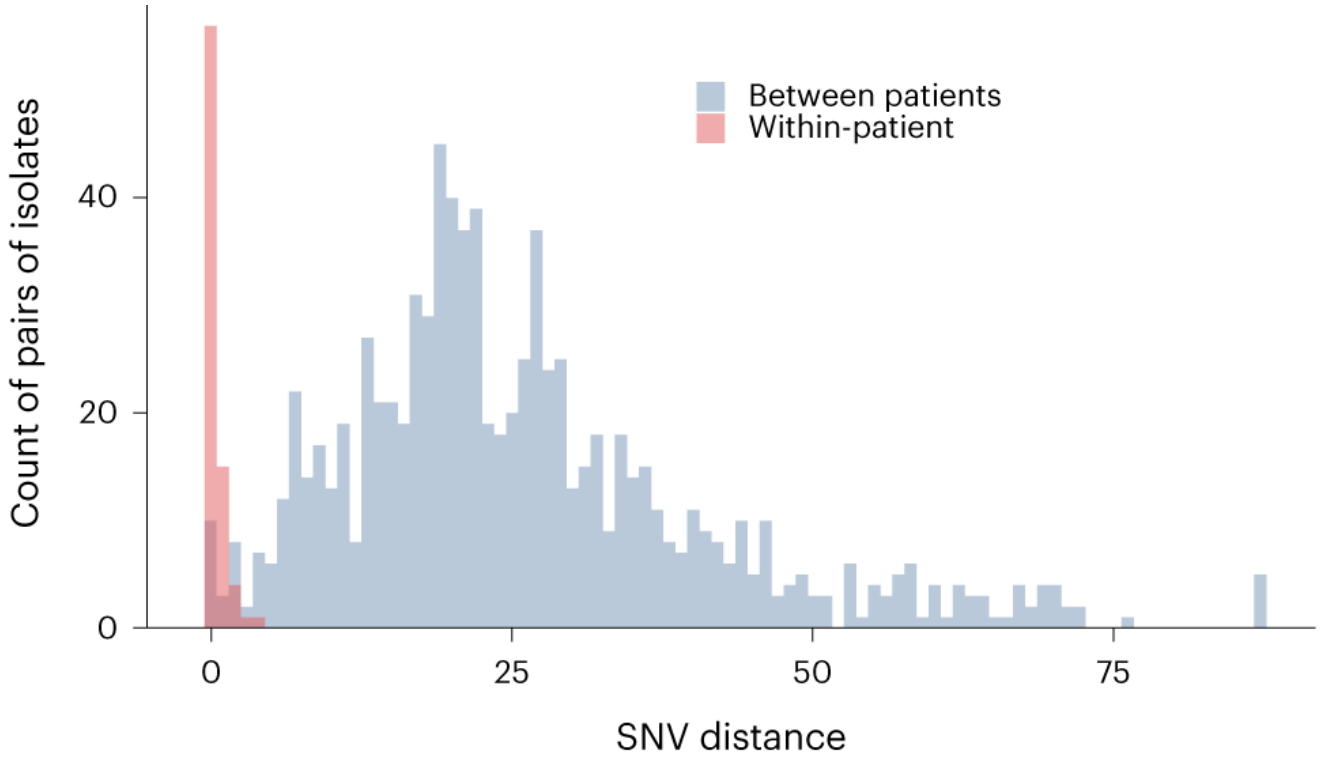
# Genomic analysis of putative culture-based within unit acquisitions

# Identifying a single nucleotide variant (SNV) threshold for detection of transmission

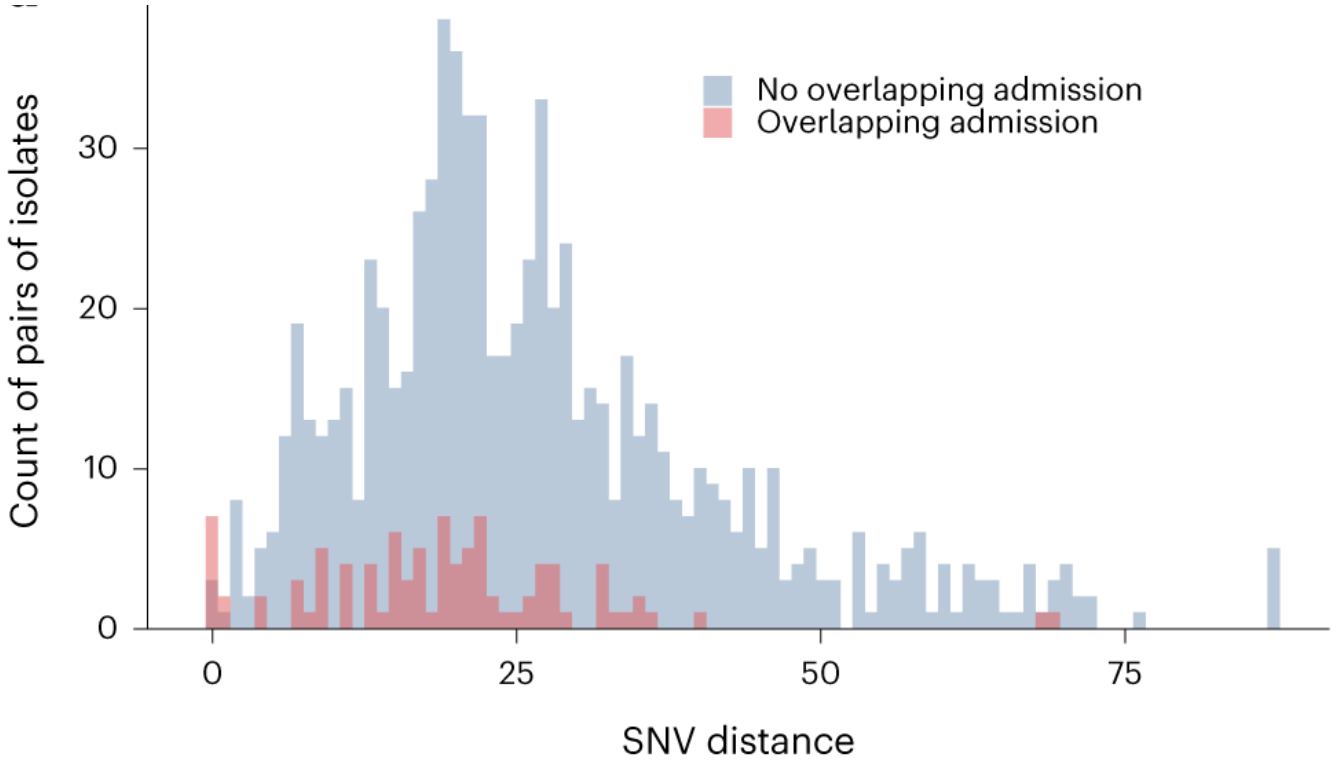
- Two common approaches to select SNV threshold, intra-patient diversity and epidemiologic enrichment
  - Maximal intra-patient diversity is used to set the upper limit on the number of SNVs detected between direct transmission pairs
  - Epidemiologic enrichment seeks to identify a threshold that maximizes consistency with shared exposures presumed to mediate transmission

- Previous work in *C. difficile* has established a 2 SNV threshold using both approaches

# More than 95% of isolate pairs of the same ST, from the same patient are 2 SNV or less

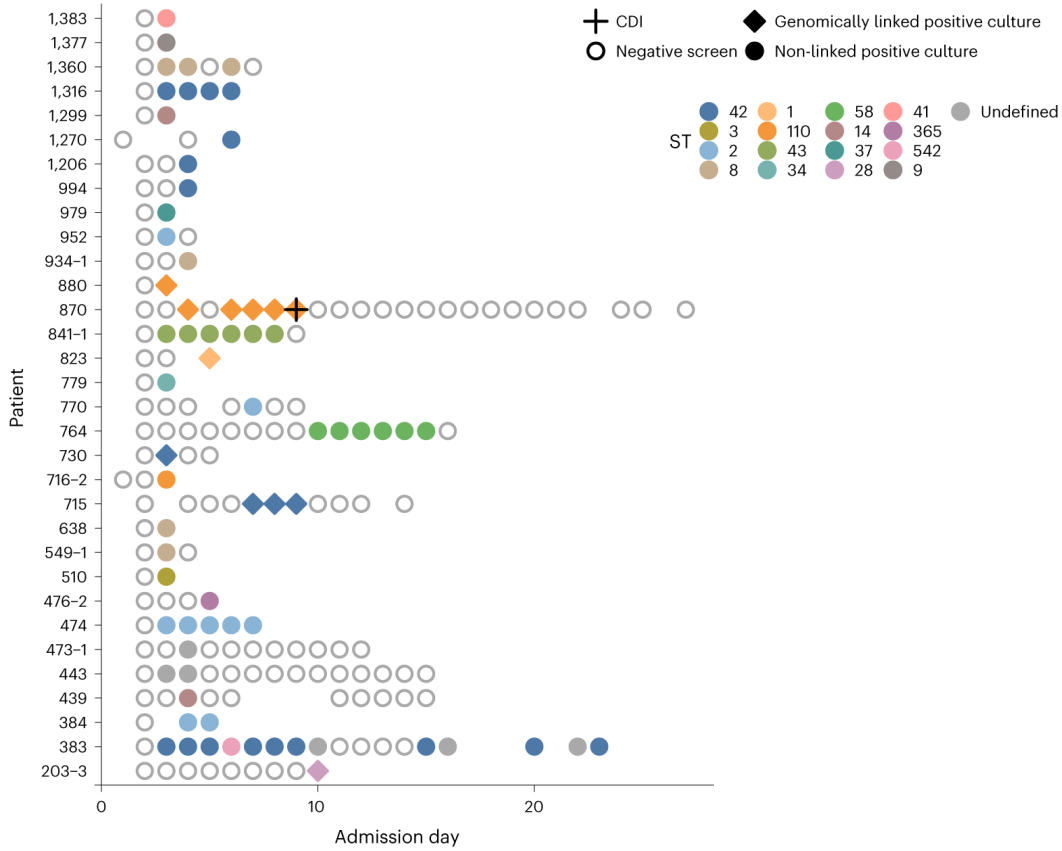


# Overlapping ICU admissions are enriched among patients whose isolates are below 2 SNVs

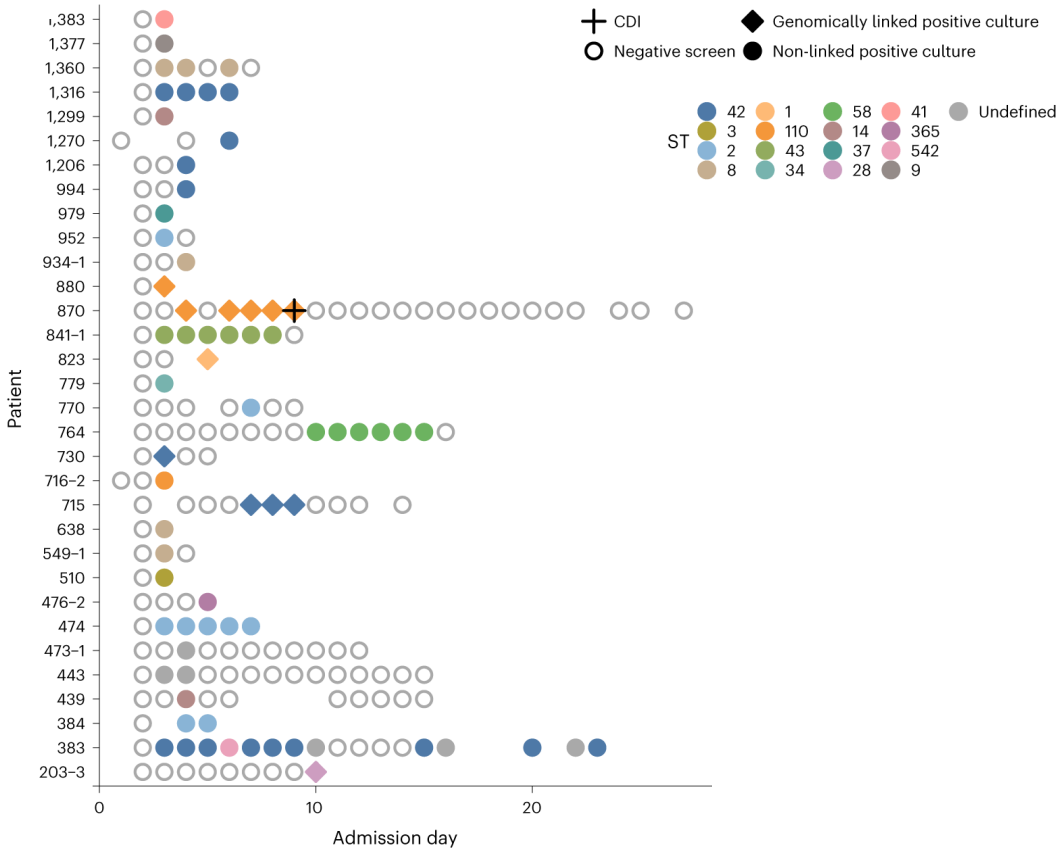


# A minority of culture acquisitions have genomic linkages to another study isolate within the MICU

- Only 7/32 acquisitions linked within 2 SNVs to another isolate
- One of these was deemed to have weak support based on small genetic distances to epidemiologically unlinked isolates from outside RUMC
- Thus, 6/32 (19%) of culture acquisitions have a putative within unit source

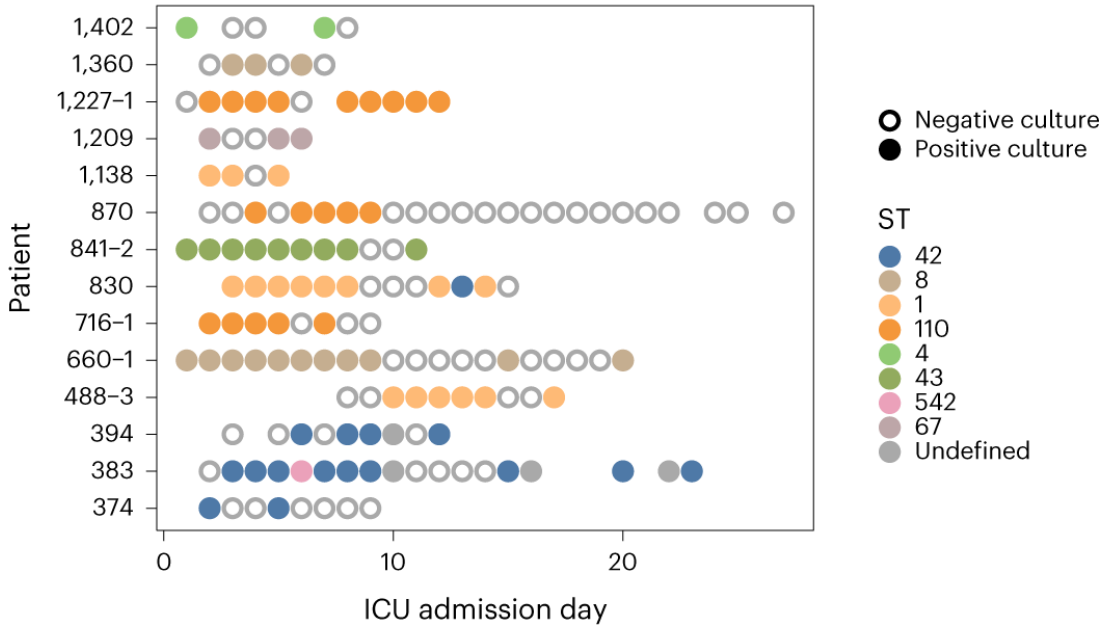


# How do we account for the other culture acquisitions that lack genomic linkages within the MICU?



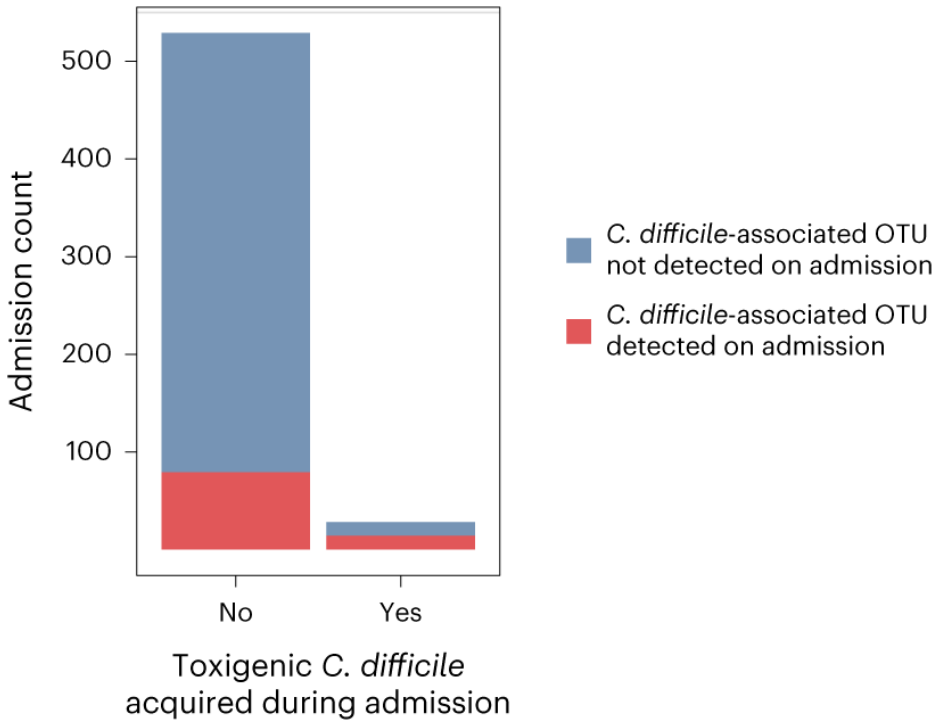

- Transmission from MICU patients not enrolled in study?
- Transmission/exposure from outside MICU?
- Persistent environmental reservoir in the MICU?
- Multiple strains carried, but only one detected?
- False negative surveillance on admission?

# Support for false negative surveillance #1: sporadic detection of *C. difficile* over time




- Among admissions with three samples, two of which were culture positive, 27/76 (36%) had at least one intervening culture negative
- Restricting to toxigenic strains, 14/36 (39%) admissions met this criteria

# Support for false negative surveillance #2: preferential detection of OTU matching *C. difficile* in admission negative samples for future acquirers

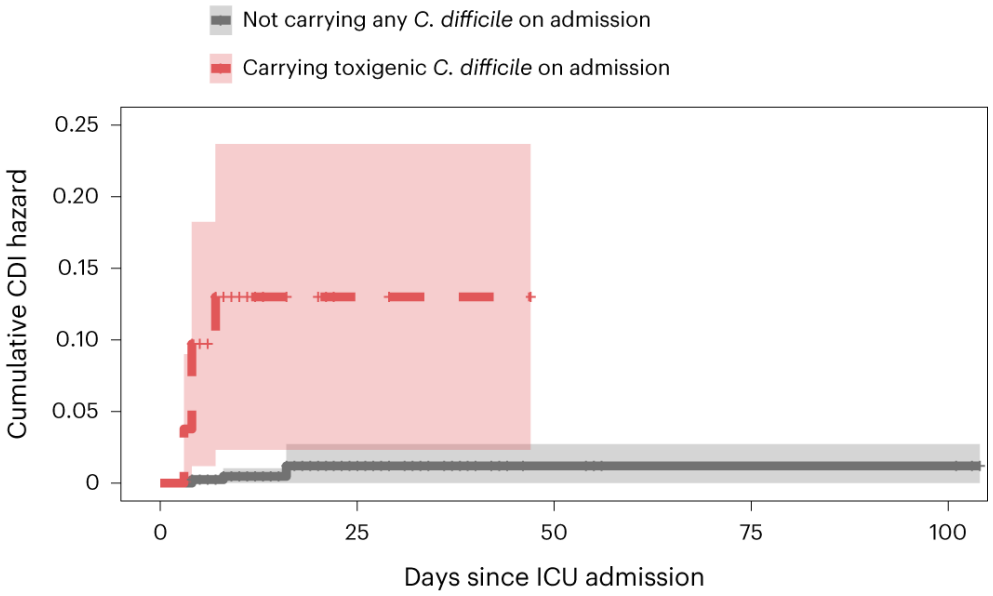



- Compared prevalence of *C. difficile* associated OTU in culture-negative admission samples from patients who did and didn't go on to culture positive for *C. difficile*

- *C. difficile*-associated OTU preferentially observed in admission samples from patients who subsequently culture positive ($p < 0.001$)

# Does colonization influence the risk for infection?

# Colonization on admission is associated with subsequent episode of hospital-onset CDI




- 11 HO-CDI cases in MICU cohort were identified based on EHR record of diarrhea and PCR for *tcdB* on day 4 of hospitalization or later
- 2 cases in patients whom we did not detect *C. difficile* on admission, but did not qualify for acquisition analysis
- 3 cases in patients with culture-based acquisition, but only 1 of which had genomic support of within unit acquisition
- 6 cases in patients who were colonized with *C. difficile* on admission (HR 24.4, 95% CI 6.89-86.5, $p < 0.001$)

# Summary and conclusions

- We identified six genomically supported cases of within MICU transmission of *C. difficile* among asymptomatic carriers over 9 months, leading to one documented case of HO-CDI
  - => suggests current infection prevention practices in the MICU are limiting within unit cross-transmission among asymptomatic carriers, given reservoir of 120 carriers

- Consistent with prior studies, we observed an >20-fold increased risk of HO-CDI in patients asymptomatically colonized with *C. difficile* on admission
  - => supports the contribution of transition from colonization to infection to HO-CDI

- Together, these findings support the contribution of asymptomatic carriage to HO-CDI in the MICU being primarily mediated by carriers themselves developing CDI, versus onward transmission to others

# Limitations

- Study limited to single MICU in single healthcare setting
- Cannot rule out hospital acquisition via other routes beyond within-unit patient-to-patient transmission among asymptomatic cases (e.g. outside unit, shared procedure rooms, HCWs, environmental contamination)
- Did not track longer term incidence of CDI among patients acquiring C. difficile in the unit
- Study results likely would not translate to times or places where epidemic healthcare associated lineages (e.g. ST1/027) are circulating at high prevalence

# Implications of these results on infection prevention

- These results support the ability to prevent cross-transmission of *C. difficile* within a unit with effective measures
  - Single rooms, cleaning with sporicidal agents
- These results support the importance of understanding triggers of the transition from colonization to infection
  - Specific antibiotics, other medications
- These results support the value of whole-genome sequencing in understanding putative transmission among colonized and infected individuals in hospitals
  - Apparent transmission clusters can be conclusively ruled out

# Acknowledgements

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