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Concise Communication

Molecular epidemiology of *Clostridioides difficile* isolates in a non-outbreak setting at a comprehensive cancer center

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Abstract

Ribotyping was performed on *Clostridioides difficile* isolates from patients with malignancies. Thirty-one (27.9%) isolates from 111 episodes of colitis were recovered representing 14 ribotypes with 25 (80.6%) belonging to 6 ribotypes (014/020, 1/VPI/077/087, 05/015, 015/046, 05/053, 106/174). We identified three novel ribotypes with 1 carrying gene encoding for binary toxin.

Keywords: *Clostridioides difficile*; molecular epidemiology; ribotyping; malignancy

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Clostridioides difficile infection (CDI) is a frequent complication of cancer care. Patients with hematological malignancies and solid tumors, in addition to receiving their care in the outpatient oncology facilities, are frequently exposed to the inpatient hospital environment. As a result, they have increased risk of colonization with *Clostridioides difficile* spores, and under the selective antibiotic pressure on the intestinal microbiome, they can develop CDI.[1] We aimed to characterize the molecular epidemiology of *Clostridioides difficile* strains causing colitis in order to better understand acquisition and transmission in a nonoutbreak setting in a cancer population.

Methods

We retrospectively studied cases of *Clostridioides difficile* colitis that occurred in patients with hematological malignancies or solid tumors at Roswell Park Comprehensive Cancer Center. Roswell Park is a 133-bed National Cancer Institute-designated Comprehensive Cancer Center in Buffalo, NY. The study was approved by Roswell Park's Institutional Review Board. The study period extended from February to December 2016.

The patients' charts were reviewed and data were collected on age, sex, underlying malignancy, and admission to intensive care unit. The presence of leukopenia (white blood cell count of $\leq 1.5 \times 10^9$/L), neutropenia (neutrophil count of $\leq 0.5 \times 10^9$/L), the level of creatinine and albumin, days of exposure to antibiotics, and proton pump inhibitors during the preceding 3 months were recorded. The incidence of CDI was estimated per 10,000 patient days of hospital admission.

Severe CDI was defined as disease associated with peripheral blood leukocytosis (white blood cell count of ≥15,000 cells/mL) and/or elevated serum creatinine (creatinine level >1.5 mg/dL).[2] Fulminant CDI was characterized by hypotension, ileus, or toxic megacolon.[2] Crude mortality was determined at 30 and 45 days after diagnosis of CDI.

Patients were tested for *Clostridioides difficile* when clinically indicated at the discretion of the treating physician. The diagnostic algorithm included initial detection of *Clostridioides difficile* Glutamate Dehydrogenase antigen and toxins A and B, using the C. Diff Quick Check Complete® immunoassay. If the *Clostridioides difficile* toxin was negative but the glutamate dehydrogenase antigen was positive, the sample was further tested by PCR using the BioFire® FilmArray® gastrointestinal panel.[2]

All patients with positive results by either *Clostridioides difficile* toxin or PCR assays were placed on "contact plus" precautions that included strict contact isolation, handwashing with soap and water, and bleach terminal cleaning.[3]

Clostridioides difficile isolation and ribotyping were performed at the Department of Pathology at Washington University, St. Louis, MO. *Clostridioides difficile* was isolated from fecal samples with a previously described CCMB-TAL broth enrichment technique[4] and identified using VITEK MS MALDI-TOF MS. Ribotyping and identification of toxins A and B were performed as previously described.[5,6] The PCR-ribotyping was evaluated for genetic similarity using the DiversiLab Bacterial Barcodes software program (bioMérieux, Durham, NC) and compared to a reference library of

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Table 1. Demographics and Basic Characteristics of 31 Patients with Typable *Clostridioides difficile* Strains

	Clostridioides difficile Infection (CDI) (n: 31)
Age	60 (8–77)
Sex (F/M)	15/16
Service	
Hematology	18 (58.1%)
LEU/LYM/TCT	5/6/7
Solid tumors	7 (22.6%)
Surgical services	6 (19.4%)
Malignancy	
MDS/AML/ALL	9 (29%)
HL/NHL/MM	9 (29%)
GI malignancies	4 (12.9%)
Breast cancer	3 (9.7%)
Urothelial cancer	3 (9.7%)
Other solid tumors[a]	3 (9.7%)
Severe CDI	6 (19.4%)
Admission to ICU	6 (19.4%)
Antibiotic usage (in days, within 3 months prior)	
b-lactam antibiotics	5.5 (0–66)
Antibiotics with anaerobic spectrum	6.0 (0–66)
Other antibiotics	5.5 (0–52)
Proton pump inhibitor usage	15 (48.4%)
White blood cell ($\times 10^9$)	5.23 (< 0.1–16.71)
Presence of neutropenia	5 (16.1%)
Creatinine	0.83 (0.34–5.25)
Albumin	3.4 (2.1–4.5)
Mortality	
30-day mortality	4 (12.9%)
45-day mortality	13 (41.9%)

Note. LEU, leukemia; LYM, lymphoma; TCT, transplantation and cellular therapy. ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; MDS, myelodysplastic syndrome; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; MM, multiple myeloma.
[a]Ovarian cancer, lung cancer, glioma.

ribotypes.[5] Three nontoxigenic *Clostridioides difficile* strains that were isolated from 3 patients with diarrhea but negative stool *Clostridioides difficile* toxin and PCR assays served as controls.

Results

The incidence of CDI was 0.29 cases per 10,000 inpatient days and remained stable throughout the study period. Of 111 episodes of CDI 66 stool samples were available and sent for isolation of *Clostridioides difficile*. Of 66 samples, 31 isolates were recovered in cultures and studied with molecular typing. The isolates corresponded to CDI in 30 patients with one patient having recurrent disease 2 weeks after completion of treatment. The demographics of 31 patients are provided in Table 1. Diagnosis was established by positive toxin and antigen assays in 19 (61.3%) cases and with PCR in 12 (38.7%) cases when toxin and antigen assays were discordant.

The 31 isolates with available typing from patients with CDI represented 12 ribotypes. Twenty-five isolates (80.6%) belonged to 6 ribotypes, represented by 3–7 strains and the rest 6 (19.4%) were unique ribotypes. The most prevalent ribotype was 014/020 (n=7, 22.6%). Other ribotypes comprised 1/VPI/077/087 (n=5 in 4 patients, 12.9%), 05/015 and 015/046 (n=4 patients each, 12.9%), and 05/053 and 106/174 (n=3 patients each, 9.7%).

Three novel ribotypes were identified (designated as Novel A, B, and C). Novel ribotype A was a nontoxigenic strain and was identified in a control sample. Novel ribotype C possessed the *cdtA* and *cdtB* genes encoding for binary toxin. None of the 2 patients with toxigenic novel ribotypes presented with severe CDI and both responded to antibiotic therapy.

The hypervirulent ribotype 078 was detected in one patient while the *cdtA* and *cdtB* genes encoding for binary toxin were present in a total of 3 isolates (1 with novel ribotype C). None of these patients presented with severe disease, fulminant CDI, or associated mortality. The 027 ribotype was not detected in our cohort.

Evaluation of genetic similarity of ribotype band patterns showed 6 lineages of 2–5 isolates among 19 (63.3%) patients (Figure 1). These lineages corresponded to 5 out of 6 most common ribotypes. The exception was isolates of ribotype 106/174 of which SI was 90% to 94.9%. Epidemiological investigation showed occasional temporal and spatial overlap among several patients within the clusters.

Discussion

The incidence of CDI among patients with malignancies is significantly higher than in the general population.[7,8] In our cohort, CDI incidence was 0.29 cases per 10,000 inpatient days. During this period the incidence of *Clostridioides difficile* colitis remained steady, a pattern consistent with a nonoutbreak setting.

Molecular typing documented shared clones in the majority of patients (80.6%) in our cohort. The most common ribotypes were 014/020, followed by ribotypes 1/VPI/77/87, 05/015, and 015/046. Ribotype 014/020 is one of the most prevalent ribotypes with worldwide distribution[9] and an emerging ribotype in the United States.[10] Less is known about the 1/VPI/77/87 ribotype that in our cohort comprised a group of 5 isolates originating from 4 patients. No isolate carried the genes for the binary toxin. They all caused healthcare-associated CDI with 4 isolates falling under the same lineage, clustering over a period of 3 months. CDI associated with this ribotype was severe in 3 cases but responded to antibiotic therapy, except for one patient who was heavily treated with antibiotics and experienced a relapse.

The hypervirulent ribotype NAP1/027 was not detected in our cohort.[7] The hypervirulent ribotype 078 was detected in one patient and the *cdt*A and *cdt*B genes encoding for binary toxin were present in another 2 patients (1 with the novel ribotype C). None of these patients presented with severe or fulminant disease. Among the 3 novel ribotypes, novel ribotype A was a nontoxigenic strain identified in a control stool sample.

Limitations of our study include its retrospective nature, the single center data source, and failure to recover all isolates for ribotyping. The failure to recover many of the isolates for ribotyping was attributed to suboptimal processing of the specimens during interstate transfer to the reference laboratory for molecular typing, which led to overgrowth of competing commensal flora. It might also reflect the superior sensitivity of

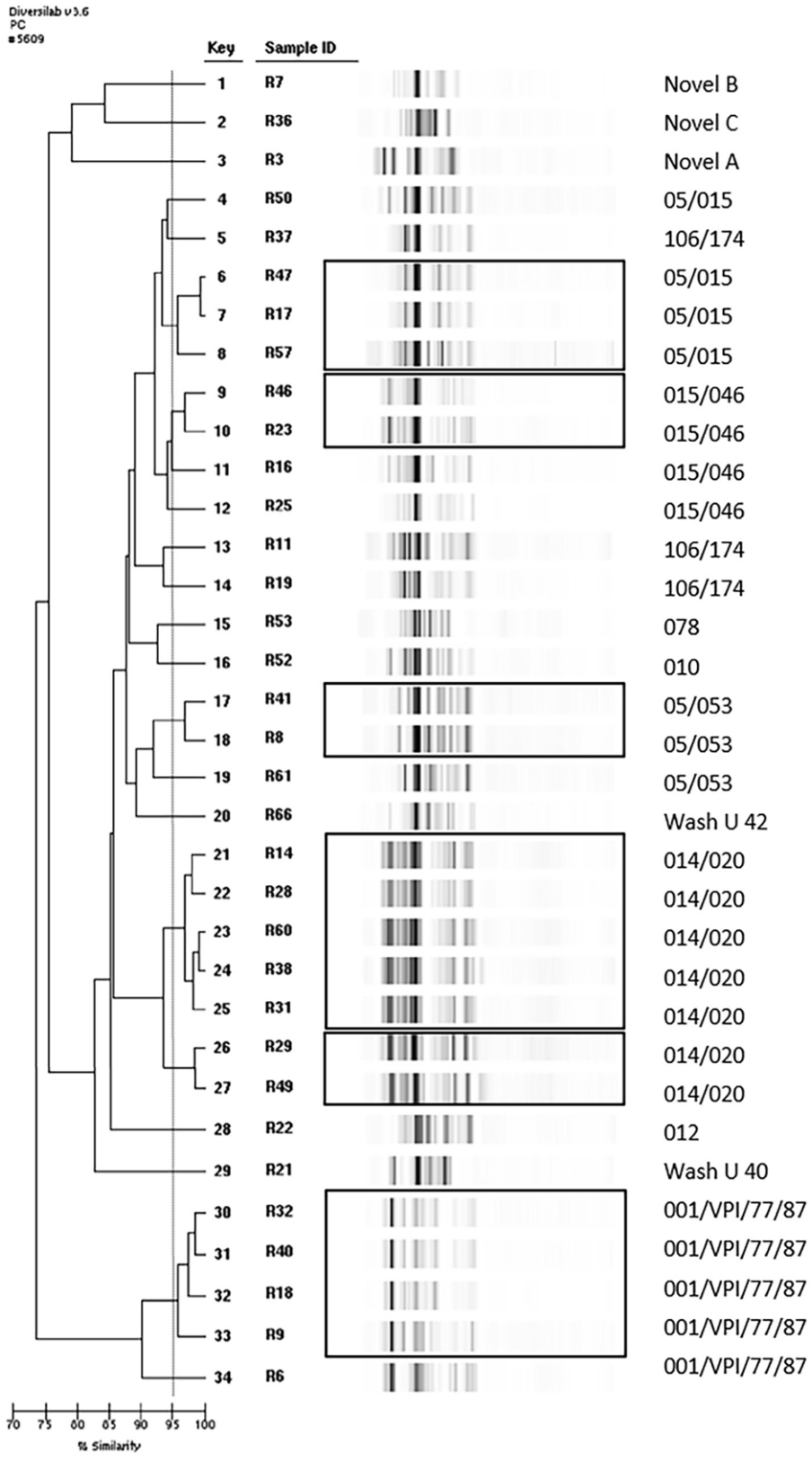

Figure 1. Dendrogram illustrating the gel electrophoresis profiles of *Clostridioides difficile* isolates. Footnote of Figure 1: [a]Isolates R3, R50, and R52 are nontoxigenic control strains. [b]Isolates R32 and R40 originate from a patient with a recurrent episode of *Clostridioides difficile* colitis.

PCR leading to detection of nonclinically significant carriage of *Clostridioides difficile* or nonviable bacterial elements.

We demonstrated that in a nonoutbreak state when incidence rate of CDI remained stable CDI was caused by several *Clostridioides difficile* lineages. Lack of a predominant strain suggests community acquisition of *C. difficile* spores and demonstrates the value of molecular analysis rather than relying solely on days of hospitalization in defining hospital-acquired *C. difficile* infection.

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