**Supplementary Material**

Transmission of *Clostridioides difficile* infection (CDI) from patients < 3 years of age in a pediatric oncology setting

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**Supplementary Figure 1: The most frequent *C. difficile* MLST sequence types in Pediatric and Adult CDI cases at study institution (2014-2017)**

**Supplementary Table 1: Donor Demographics/Total days admitted**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Donor Cluster** | **Age (yrs)** | **ST** | **Underlying diagnosis** | **Chemo in 30*d*** | **Abx in 30*d*** | **Diarrheal stools documented** | **LOS of index CDI hospitalization (days)**  **(median = 7*d*; average = 37*d*)** |
| **1** | **2** | **2** | **Retinoblastoma** | **Yes** | **Yes** | **No** | |  | | --- | | **7** | |
| **2** | **1** | **2** | **Neuroblastoma** | **Yes** | **Yes** | **Yes** | **5** |
| **3** | **2** | **42** | **ALL** | **Yes** | **Yes** | **No** | **84** |
| **4** | **1** | **110** | **Neuroblastoma** | **Yes** | **Yes** | **No** | **5** |
| **5** | **2** | **3** | **Primary immunodeficiency (DOCK 8)** | **No** | **Yes** | **Yes** | **127** |
| **6** | **1** | **8** | **Retinoblastoma** | **No** | **Yes** | **No** | **3** |
| **7** | **2** | **37** | **Primary immunodeficiency/DLBCL** | **No** | **Yes** | **Yes** | **28** |

ALL = acute lymphoblastic leukemia; Chemo = chemotherapy; *d* = days; DLBCL = diffuse large B-cell lymphoma; DOCK8 = Dedicator of Cytokinesis 8; LOS = length of stay; ST = sequence type

**Supplementary Table 2:** Stool Consistency and symptoms for included patients as extracted from electronic health record.

|  |  |  |  |
| --- | --- | --- | --- |
| Stool Constancy | Liquid n=8 | Semiformed n=28 | Formed n= 3 |
| Diarrhea | Yes = 24 | No=15 |  |
| Laxative use within 48hrs hours | Yes= 6 | No=33 |  |

**Supplementary Methods:**

**MLST banking and CD culture**: Stools were thawed, and ethanol shocked with a 1:4 dilution in 100% ethanol for at least 1 hour. After incubation and centrifugation, samples were inoculated on selective media for the detection of *C. difficile* and incubated anaerobically. Growth confirmation of a single colony by PRO disk was performed with the remaining portion of the colony subbed to a blood agar plate for isolation. After a 48-hour anaerobic incubation, samples were submitted for whole genome sequencing.1

**WGS**: Genomic DNA was extracted from each isolate using the QIAamp Genomic DNA kit (Qiagen). WGS was performed using the Nextera XT Sample Prep Kit and paired-end sequencing (150 bp ×2) on the NextSeq 550 platform (Illumina). Multi-locus sequence typing (MLST) was identified by MLST algorithm (https://github.com/tseemann) after *de novo* sequence assembly using SPAdes (v3.9.0). SNPs were identified using Snippy (v3.2, https://github.com/tseemann) with alignment and mapping to R0104 (ST1) complete genome (NCBI accession number: CP025044) as reference. Phylogenetic tree and pair-wise distance matrix were generated from CoreSNP analysis of Snippy.

**1.** Sim JH, Anikst V, Lohith A, Pourmand N, Banaei N. Optimized Protocol for Simple Extraction of High-Quality Genomic DNA from Clostridium difficile for Whole-Genome Sequencing. *J Clin Microbiol* 2015;53:2329-2331.