

***CLOSTRIDIUM DIFFICILE* INFECTION AND COLONIZATION IN PAEDIATRIC
ONCOLOGY PATIENTS**

By

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ABSTRACT

Clostridium difficile infection (CDI) is the most common hospital-associated infection and is linked to increased morbidity, mortality and costs. Asymptomatically colonized patients may act as an infection reservoir and their numbers have been found to exceed symptomatic CDI cases. In addition to higher rates of CDI among children hospitalized with cancer compared to those without an oncologic diagnosis, these patients also experience substantially higher *C. difficile* colonization rates. However, the current published literature does not adequately address the natural history of *C. difficile* colonization in this population, in terms of who is at greatest risk for developing colonization, duration of colonization, or progression to CDI.

A retrospective longitudinal cohort study of pediatric oncology patients admitted to the oncology ward at McMaster Children's Hospital (MCH) was conducted from September 1 2016 to February 28 2018. Patients who were routinely screened for antibiotic-resistant organisms (AROs) upon admission per hospital policies had their stored samples subsequently tested for asymptomatic carriage of *C. difficile*. A retrospective analysis was completed to determine predictors of colonization and risk factors for progression to subsequent CDI.

We observed a lower colonization rate than other studies have reported in the literature. Duration of colonization was likely brief and none of the colonized patients subsequently developed CDI. There were no statistically significantly associated predictors for asymptomatic colonization when colonized patients were compared to those who were never colonized.

In conclusion, it remains to be determined which patients in the pediatric oncology population are at highest risk for *C. difficile* colonization, and which colonized patients may be more likely to progress to CDI. Future studies assessing *C. difficile* colonization in this population would benefit from standardization of microbiological testing methods for determining colonization, prospective study approaches, larger cohort sizes, and testing in both the inpatient and outpatient settings.

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ABBREVIATIONS

ARO	Antibiotic resistant organism
CDI	<i>Clostridium difficile</i> infection
CPE	Cytopathic effect
DNA	Deoxyribonucleic acid
EIA	Enzyme immune assay
ELFA	Enzyme-linked fluorescent antibody assay
ELISA	Enzyme-linked immunosorbent assay
GDH	Glutamate dehydrogenase
GEE	Generalized estimating equations
H2RA	Histamine ₂ -receptor antagonist
IPAC	Infection prevention and control
MCH	McMaster Children's Hospital
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NAAT	Nucleic acid amplification test
PCR	Polymerase chain reaction
QICC	Corrected quasi-likelihood under independence model criterion
SLR	Simple logistic regression
VRE	Vancomycin-resistant Enterococcus

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PART I: INTRODUCTION

Clostridioides difficile (formerly *Clostridium difficile*) is an obligate gram-positive anaerobic bacterium that can persist in the environment by forming spores¹. It is found in water, soil, meats and vegetables, gastrointestinal tracts of animals and humans, however it is also particularly common in health care settings^{2,3}. Their spores are physically robust and are stable to oxygen stress, temperature extremes and desiccation, thus playing an important role in the epidemiology and transmission of these bacteria in the hospital environment as they can be difficult to eradicate¹.

C. difficile has been established as the most common infectious cause of antibiotic-associated diarrhea, accounting for about 25% of all cases and is the underlying cause in nearly all cases of severe disease⁴. In addition, *C. difficile* infection (CDI) is the most common hospital-associated infection and is linked to increased morbidity, mortality and costs^{5,6}. However, asymptomatic *C. difficile* colonized patients may act as an infection reservoir and the number of colonized patients has been found to exceed symptomatic CDI cases among hospital patients⁷. Unlike CDI that has a well-established formal case definition including clinical and microbiological criteria^{8,9}, asymptomatic carriage with *C. difficile* is less well defined⁷. Furuya-Kanamori et al. have proposed defining *C. difficile* colonization as the absence of diarrhea (or if present, attributable to a cause other than CDI) without colonoscopic or histopathologic findings consistent with pseudomembranous colitis, and either the detection of *C. difficile* or the presence of *C. difficile* toxins⁷.

There has been a growing interest in alternative sources of *C. difficile* beyond patients with CDI and the hospital environment. Recent studies have reported that a large proportion of CDI cases

are not due to transmission from known CDI cases, and that asymptomatic carriers can also transmit the disease³. Using various sequencing technologies, only 25-50% of patients with symptomatic CDI can be linked to a previously identified CDI patient^{10,11}. With the ongoing global burden of CDI, including the associated morbidity, mortality and health care costs, a better understanding of *C. difficile* colonization and the factors that contribute to the progression and prevention of disease is warranted.

BIOLOGY OF *C. DIFFICILE*

C. difficile was originally identified by Hall and O'Toole in 1935 as a component of the normal colonic flora of newborn infants¹². They also showed that the cell-free supernatants of broth cultures were highly lethal to a variety of experimental animals, defining the biological activities of the bacteria's cytotoxins. It is now known that the clinical disease and pathogenicity of *C. difficile* is attributable to the production of cytotoxins. The majority of *C. difficile* strains produce both toxins A (*tcdA*, an enterotoxin) and B (*tcdB*, a cytotoxin), however a third toxin, binary toxin, has been identified among recent epidemic strains where outbreaks have been associated with increased severity and mortality¹. The toxins enter the cytoplasm of the colonocytes by binding to receptors found on the luminal-facing side of these cells. Within the cells they inactivate various proteins and trigger apoptosis of the cells, resulting in an acute inflammatory reaction that leads to diarrhea and colitis¹³.

CLINICAL PRESENTATION

The spectrum of disease caused by *C. difficile* is broad, ranging from asymptomatic colonization, to *Clostridium difficile* infection (CDI) that typically presents with diarrhea but can occasionally result in toxic megacolon and death¹⁴.

There is an increasing burden of CDI in the paediatric population both in incidence of CDI among hospitalized patients and hospitalizations attributable to CDI¹⁵. Furthermore, the incidence of CDI has been reported to be more than 15-fold higher among children hospitalized with cancer compared to those without cancer¹⁶. Risk factors for developing CDI among children with cancer are thought to be similar to those seen in adults with cancer: increased contact with the healthcare system (admission to hospital, outpatient clinics), immunosuppression with chemotherapy, and exposure to broad-spectrum antibiotics¹⁷.

HUMAN SOURCES OF CDI

Patients can shed *C. difficile* during diarrheal episodes as well as following completion of therapy when symptoms have stopped. Sethi et al. were able to culture the bacteria from stools of half of adult patients up to 4 weeks after they had completed treatment for CDI. In addition, the authors found skin and environmental contamination persisted for the same duration¹⁸.

Though it does not contribute to the current morbidity of a patient, asymptomatic carriage contributes to the epidemiology of *C. difficile* infection as it may contribute to transmission and CDI in others^{19,20}. Asymptomatic carriers and the associated shedding of spores remains undetected due to lack of routine screening. Genotyping has revealed almost one third of CDI cases could be linked to asymptomatic *C. difficile* carriers²⁰. Those who are colonized at the time of admission also appear to contribute to the sustained transmission of *C. difficile* on inpatient adult wards^{19,21}.

Both simulation models and clinical data have demonstrated that screening and isolating *C. difficile* carriers can result in a reduction in the CDI incidence and could potentially be a cost-effective intervention²²⁻²⁵. One clinical study by Longtin et al. has prospectively evaluated the effectiveness of isolating asymptomatic carriers. Patients were screened at the time of admission, and if found to be colonized they were placed under contact precautions during their hospitalization. The authors found the incidence of CDI decreased significantly after the intervention compared to the pre-intervention period²³. There are currently no pediatric studies assessing colonization and subsequent transmission.

EPIDEMIOLOGY OF COLONIZATION

Colonization refers to the detection of an organism in the absence of clinical symptoms of infection¹. Diarrheal symptoms can be attributed to multiple infectious and non-infectious etiologies, thus making the distinction between *C. difficile* colonization and infection challenging. Among published studies, there is considerable variability in the definition of colonization based on number of microbiological samples obtained, and the time period during which there needs to be an absence of clinical symptoms³. Additionally, different laboratory methods for detecting the presence of either *C. difficile* (either toxigenic or non-toxigenic) or its toxin affect the reported incidence rates of both CDI and colonization¹⁴.

Asymptomatic colonization rates among neonates and infants are higher than that found in the general population, ranging up to over 70%²⁶⁻²⁹. Rates subsequently decrease to roughly 10% during the first year of life^{26,30}, and continue to decrease thereafter before reaching rates comparable to healthy adults by the age of 2 to 3 years³⁰⁻³². Recent reviews of asymptomatic *C. difficile* colonization among healthy adults report rates between 4 to 15%^{3,33}. However, there is

substantial variability depending on colonization with toxigenic versus non-toxigenic strains, testing patients at the time of admission versus during hospitalization, or when evaluating previously healthy adults versus patients admitted to long term care facilities. One recent pediatric *C. difficile* narrative review that included studies assessing colonization among children up to 13 years of age reported pooled asymptomatic colonization rates in this age group ranging from 0 to 15%³⁴. However, it is also important to highlight that substantially higher colonization rates have been reported in the paediatric oncology population (affecting up to 25%-40% of patients)^{35,36}.

DEVELOPMENT OF COLONIZATION AND SUBSEQUENT PROGRESSION TO CDI

In addition to the risk of transmitting *C. difficile*, development of CDI in a *C. difficile* carrier is another concern in carriers. After being exposed to bacterial spores, key mechanisms that underlie acquiring or resisting colonization involve the host's gut microbiota and the host immune response against *C. difficile*. The gut microbiota plays an important role throughout the whole life cycle of *C. difficile*, from germination and colonization to the subsequent establishment of symptomatic disease³. The presence of certain bacteria in the human colon affects the balance between primary and secondary bile salts, which stimulate and inhibit the germination process respectively³⁷. The microbiota may also provide direct resistance mechanisms as they compete for niches and nutrients, and produce antimicrobial substances^{38,39}. Compared to patients with CDI where studies have described both a lower species richness and lower microbial diversity, the alterations in gut microbial composition in *C. difficile* carriers are less well described⁴⁰.

C. difficile toxins A (*tcdA*) and B (*tcdB*) play an important role in eliciting the intestinal inflammatory response in CDI that results in neutrophil infiltration into the gut mucosa, and subsequent induction of adaptive immunity⁴¹. Specifically, the antibody-mediated response has

been thought to mediate the adaptive immunity against *C. difficile* colonization and CDI. Antibodies against *C. difficile* surface proteins have been isolated from asymptomatic carriers⁴² and *in vitro* studies appear to demonstrate a protective role against colonization³. Conversely, antibodies to *tcdA* and *tcdB* appear to protect against clinical disease and likely therefore the progression from colonization to CDI⁴³.

Rates of *C. difficile* colonization exceed rates of CDI⁷ and asymptomatic carriage of *C. difficile* poses a risk for subsequent development of symptomatic disease^{7,44,45}. Two meta-analyses provide conflicting evidence for whether *C. difficile* colonization is protective or a risk factor for developing subsequent CDI. The first meta-analysis was published in 1998 and found a lower risk for developing CDI⁴⁶. The four included studies varied with regards to inclusion of nontoxigenic and toxigenic strains and the timing of determining colonization. Furthermore, the studies were all performed prior to the emergence of hypervirulent *C. difficile* strains. A subsequent meta-analysis aimed to focus on patients who were colonized at admission with toxigenic strains only⁴⁴. There was heterogeneity among the nine included studies, as samples were not always successfully obtaining within 48-72 hours of admission, some patients were admitted to a rehabilitation facility after admission to an acute care facility, and colonization was not always clearly differentiated from CDI. Nonetheless, recent studies appear to point to an increased risk, with an almost 6-fold higher risk for patients colonized with toxigenic *C. difficile* at admission to progress to CDI compared to non-colonized patients⁴⁴.

RISK FACTORS FOR COLONIZATION

Recently published literature in adult populations has identified different risk factors for colonization in the community or at admission to a hospital and risk factors for acquiring

colonization during hospital admission³. At the time of admission, epidemiological and clinical risk factors for colonization include recent hospitalization (ranging from 3 to 12 months prior), chronic dialysis, corticosteroid/immunosuppressant use, gastric acid suppression and antibodies against toxin B^{10,47,48}. Conversely, previous hospitalization in the last 2 months, use of proton pump inhibitors or H₂ blockers, or chemotherapy or cephalosporins were significant risk factors for becoming colonized during hospital admission^{49,50}. However, none of these studies focus on the oncology patients.

In their narrative review, Enoch et al. summarized case-control studies that compared pediatric patients with and without diarrheal symptoms³⁴. *C. difficile* was isolated from 72 of 438 asymptomatic patients (16.4%), with the asymptomatic carriage rate from individual studies ranging from 0% to 19%⁵¹⁻⁵⁵. The numbers from each study were small and there were conflicting results with regards to the impact of antibiotic use on clinical symptoms⁵¹⁻⁵⁴. Only one of the included studies focused on pediatric oncology patients⁵¹.

There is a paucity of literature examining risk factors for *C. difficile* colonization in the oncology population. Given that some of the highest colonization rates have been reported in this population, the potential for asymptomatic carriers to serve as reservoirs for *C. difficile* transmission, and increased burden of CDI in immunocompromised patients, a better understanding of *C. difficile* colonization in the paediatric oncology population is warranted.

CONCLUSION

The potential for *C. difficile*-colonized patients as important unexplained reservoirs for *C. difficile* transmission has led to the investigation of colonization rates among different populations and the

determination of risk factors for colonization. The literature to date has focused primarily on adult patients. It is unknown if these observations would be applicable to the pediatric oncology population, where colonization rates have been observed to be higher. Therefore, a separate review of the literature focusing on this patient group is warranted and presented in Part II. In addition, the main project in this thesis project, a retrospective cohort study, was conducted in order to determine if *C. difficile* colonization in the pediatric oncology population is a risk factor for subsequent symptomatic CDI and to identify risk factors associated with colonization at the time of admission. The findings from this thesis will be of interest to those caring for children with oncologic diagnoses in hospital including pediatric oncologists, pediatric infectious diseases specialists, and infection control practitioners.

PART II: SCOPING REVIEW

INTRODUCTION

Numerous reviews on *C. difficile* colonization have focused on epidemiological and clinical risk factors in adults^{3,7}. These included hospitalization within the last 12 months, exposure to corticosteroids, use of chemotherapy, proton pump inhibitors or H₂ receptor antagonists, prior CDI, renal disease, and previous antibiotic use^{47-49,56}. Colonization with *C. difficile* (both toxigenic and non-toxigenic) has been well described in the young infant population^{26-29,57}. However, little is known about the risk factors for asymptomatic *C. difficile* carriage and its natural history in children outside of the neonatal period^{15,30,31,58}. Given the significant morbidity associated with CDI in the paediatric oncology population⁵⁹ and our evolving understanding of the implications of asymptomatic carriage, there is a need to identify those at highest risk for colonization, and determining if colonization poses a risk for developing subsequent CDI or transmission to other patients.

A preliminary search of the Medline and EMBASE databases using the search terms “*Clostridium difficile*”, “pediatric”, and “colonization” did not reveal an existing systematic or scoping review on this topic. One narrative review was identified, however it included only one study that focused on the pediatric oncology population²⁸. Therefore, we conducted a scoping review to summarize current knowledge pertaining to *C. difficile* colonization among pediatric oncology patients and to identify gaps in the scientific literature.

The specific review questions included:

1. What is the prevalence of *C. difficile* colonization among pediatric oncology patients beyond infancy, i.e. patients >12 months of age
2. What is the natural history of *C. difficile* colonization among pediatric oncology patients
3. What are risk factors for *C. difficile* colonization among pediatric oncology patients beyond infancy?

METHODS

We used scoping review methodology to examine the extent, range and nature of research activity in this topic, as well as identify research gaps in the existing literature⁶⁰. The methodological framework proposed by Arksey and O'Malley⁶⁰ and further developed by Levac et al.⁶¹ was used.

It progresses through the following six stages:

1. Identifying the research question – Scoping study questions are broad. However it is important to consider the concept, target population, and health outcomes of interest to clarify the focus of the scoping study. The purpose of the study should be linked with the research question.
2. Identifying relevant studies – Similar to systematic reviews, this step involves systematically searching through a variety of sources including electronic databases, reference lists, hand-searching of key journals, and the grey literature.
3. Study selection – This stage should be considered an iterative process involving searching the literature, refining the search strategy and reviewing articles for study inclusion. At least two reviewers should independently review abstracts for inclusion, as well as full articles for inclusion. When disagreements on study inclusion occur, a third reviewer can determine final inclusion.

4. Charting the data – A data-charting form should be collectively developed and piloted, in order to determine which variables to extract in order to answer the research question. Charting should be considered an iterative process in which researchers continually extract data and update the data-charting form.
5. Collating, summarizing and reporting the results – There should be three distinct steps. First, the analysis which would include descriptive numerical summary analysis and qualitative thematic analysis. Second, a report of the results and outcomes that refers to the overall purpose or research question. Finally, a consideration of the implications of findings for future research, practice and policy.
6. Consultation – Incorporate opportunities for knowledge transfer and exchange with stakeholders in the field.

Data Sources and Search Strategy

We searched the Cochrane Infectious Diseases Groups Specialized Register (April 2018), the Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2018, Issue 4), MEDLINE® Epub Ahead of Print, In-Process & Other Non-Indexed Citations (May 17, 2018), MEDLINE® Daily (May 17, 2018), MEDLINE® (1946 – May 17, 2018), EMBASE (1947 – May 17, 2018), CINAHL (1981 – May 17 2018), and Web of Science (1900 – May 17 2018). The reference lists of identified reviews, clinical practice guidelines and the final included studies were reviewed for additional citations. A comprehensive search strategy including the search terms “*Clostridium difficile*”, “*pediatrics*”, “*oncology*” and “*cancer*” was developed with the assistance of a librarian experienced in conducting systematic and scoping reviews. (*Appendix I*) A limit was set for English language studies only.

Inclusion Criteria

Types of Studies

This review included all types of studies that reported on pediatric oncology patients, including randomized controlled trials and observational studies including case reports and case series.

Types of Participants

Studies that enrolled pediatric oncology patients between 1 and 18 years of age were considered for inclusion. Studies that included infants less than one year of age or participants over 18 years of age were included if these age groups comprised less than 25% of the study population, or if authors were able to provide patient-level or disaggregated data about the age group of interest.

Defining Colonization

In addition to clearly stating an absence of diarrhea (or if present, attributable to a cause other than CDI), *C. difficile* colonization needed to be confirmed by microbiological methods (e.g. stool culture, toxin gene detection (e.g. polymerase chain reaction (PCR) or nucleic acid amplification test (NAAT), toxin enzyme immune-assay (EIA), cytotoxin assay, or glutamate dehydrogenase (GDH)). Studies only enrolling participants with active infection (i.e. fulfilling the clinical diagnostic criteria for CDI) were excluded.

Measures of Interest

Articles that reported on the burden of *C. difficile* colonization (including incidence, prevalence, measures of morbidity and mortality), risk factors for colonization, and natural history of colonization (including time to clearance of colonization, development of clinical disease with *C. difficile* infection) were considered.

Exclusion Criteria

For studies where some of the above inclusion criteria are met, we first attempted to contact authors for patient-level data. If we were unsuccessful, studies were excluded.

Study Selection

We screened all titles and abstracts identified by the initial search strategy in duplicate against the selection criteria, and deemed as ‘include’, ‘exclude’, ‘uncertain’. For titles and abstracts marked as ‘include’ or ‘uncertain’, the corresponding full text articles were retrieved and reviewed for inclusion.

Data Abstraction

Data abstraction was completed by a single reviewer. All included studies were reviewed and data collected using a pilot-tested data abstraction form. The following data was extracted when available: author(s), year of publication, publication status, study design (e.g. randomized controlled trials, observational, qualitative, commentary), country of origin, aims/purposes, study population (number of patients, age, gender, oncologic diagnoses), methodology/methods, intervention and comparator type (if applicable), outcomes, and key findings that relate to the scoping review questions. (*Appendix 2*)

Analysis

A numerical summary of the number, type and distribution of included studies was prepared. Categorical variables were reported as counts with percentages and continuous data was reported as means (with standard deviation) or medians (with interquartile range).

The narrative summary included four elements. First, a theoretical model was developed to outline what leads to *C. difficile* colonization in the paediatric oncology population, and what contributes to either clearance of or progression to symptomatic infection. Second, a preliminary synthesis was prepared to organize the findings that address the specific research questions. This included describing the direction and size of effects observed with any interventions or patient characteristics. Third, the robustness of the synthesis was assessed in order to draw conclusions about the different variables that impact *C. difficile* colonization and progression to symptomatic infection. Last, gaps in the literature were highlighted.

RESULTS

Study Selection

The search of electronic databases retrieved 760 articles, and review of references lists yielded an additional 8 articles. Following title and abstract screening, 35 articles underwent full text review. Six studies were selected and included for data extraction and quality assessment. (See *Figure 2*)

Study Characteristics

A total of 8 publications representing 6 studies were identified^{36,51,62-67}. They were all observational studies, with 5 being conducted prospectively. All studies were single-centre studies, conducted in the United States, Australia, United Kingdom, Iran, Italy and Sweden. Study dates ranged from 1982 to 2012; 3 studies were conducted in the 1980s prior to the emergence of hypervirulent strains and recognition of community-associated CDI^{63,64,67}, followed by 1 study in each of the following decades^{36,51,62}. Only one study took place in the outpatient setting⁶⁴. In the

inpatient setting, 4 studies specifically assessed colonization at the time of admission^{36,62,63,67}.

(Table 1)

Overall, demographic details were incomplete. From the descriptions provided, a broad spectrum of patients were represented including those of varying age groups up to and including the age of 18 years, those with different underlying oncologic diagnoses, and those who had previously been infected with *C. difficile*. Average or mean age as well as age ranges were reported in three studies^{36,51,64}, and one additional study reported the number of children aged less than 3 years⁶⁷. Gender was reported in two studies^{62,64}. One study only included patients with acute lymphoblastic leukemia⁶⁴, and another study specified the proportion of patients with leukemia versus solid tumors⁶⁷. Authors will be contacted to request patient level data to facilitate formal descriptive analysis of patient characteristics.

All studies utilized fresh stool samples to screen patients for *C. difficile* carriage. Five of the studies confirmed the presence of *C. difficile* using selective culture methods, and also reported on toxigenic strain detection using a variety of methods including cytopathic effect (CPE) assays^{51,62}, enzyme-linked immunosorbent assay (ELISA)⁶² for toxin A and B detection, enzyme-linked fluorescent antibody assay (ELFA)⁵¹ for toxin A detection, and cytotoxicity assays^{64,67}. The most recently conducted study utilized PCR technology for detection of Toxin B gene, and therefore only reported on the presence of toxigenic *C. difficile*³⁶. (Table 1)

Prevalence of *C. difficile* Colonization

All studies reported data to calculate the prevalence among individual patients^{36,51,62-64,67}. Overall, the colonization rate was 86/365 (23.6%) tested patients, ranging from 5/63 (7.9%)⁶³ to 17/44

(38.6%)⁵¹. Among studies that differentiated between toxigenic and non-toxigenic strains, toxigenic strains made up 60/80 (75.0%) of isolates, ranging from 2/11 (18.2%)⁶⁷ to 36/38 (94.7%)⁶² between studies. Among patients who were screened at the time of admission, 55/306 (18.0%) were asymptotically colonized^{36,62,63,67}. Of the isolates that were differentiated, 38/49 (77.6%) patients carried toxigenic strains^{62,67}. One study assessing colonization at admission used PCR for toxin B gene detection, hence there was no information on carriage of non-toxigenic *C. difficile*³⁶.

Natural History of *C. difficile* Colonization

Chiesa et al. conducted a study in the outpatient setting, where they obtained weekly stool samples from patients with acute lymphoblastic leukemia undergoing maintenance chemotherapy⁶⁴. Of the 4 patients who asymptotically carried *C. difficile* during the study period, none went on to develop diarrheal illness during the 15-week study period.

Two studies reported on *C. difficile* detection (including both culture-based or toxin-based detection) among patients who were previously diagnosed as having CDI^{36,63}. However, the authors did not differentiate between symptomatic versus asymptomatic episodes upon subsequent detection.

Risk Factors for *C. difficile* Colonization

Four studies reported on the statistical analysis of risk factors associated with *C. difficile* colonization^{36,51,62,67}. Burgner et al. reported younger age and shorter hospital stay as risk factors significantly associated with carriage of toxigenic strains⁵¹. In addition to noting colonized patients were only among those with previous hospitalizations, Dominguez et al. reported a non-

statistically significant relationship between colonization and overall greater antibiotic exposure (i.e. number of days receiving antibiotics during the preceding 12 weeks) and healthcare exposure (i.e. number of independent outpatient visits during preceding 12 weeks)³⁶. The other authors did not observe a significant relationship between colonization and age^{62,67}, gender⁶², or overall antibiotic exposure^{62,67}. The authors did not report measures of associations (such as odds ratios and relative risks) or standard deviations in these studies, and therefore pooled analyses were not conducted.

DISCUSSION

To our knowledge, this is the first scoping review on *C. difficile* colonization among pediatric oncology patients. Overall, the average prevalence of asymptomatic carriage was almost 25%, however this ranged from under 10%⁶³ to almost 40%⁵¹ depending on the individual study. When specifically tested, 75% were toxigenic strains but this ranged substantially from less than 20%⁶⁷ to 95%⁶² of tested isolates. Some studies identified age, healthcare exposure and antibiotic use as risk factors for colonization, whereas others did not^{36,51,62,67}. Only one study looked at the natural history of *C. difficile* colonization and found that patients were only briefly colonized, and none developed CDI⁶⁴.

C. difficile colonization rates ranged from under 10%⁶³ to almost 40%⁵¹, with the isolation of toxigenic strains ranging from less than 20%⁶⁷ to 95%⁶² of tested isolates. All studies except for one used culture methods for *C. difficile* detection but a variety of toxin detection methods, which can impact the reported detection rates and potentially explain the variability in detected toxigenic strains. The best standard for laboratory identification of toxigenic *C. difficile* has yet to be established¹⁴. For the last few decades, the two primary reference tests have been the *C.*

difficile cytotoxin neutralization assay (CCNA) for cytopathic effect (CPE), which has excellent specificity and correlates well with clinical disease, and toxigenic culture (TC), which has higher sensitivity but lower specificity. However, these tests have limited availability, are costly, labour-intensive and usually reserved for non-clinical applications^{9,14}. Identification of the toxin by enzyme immunoassay (EIA) was thought to be a pragmatic approach to diagnosing clinical disease, and toxin A&B EIAs were widely used because of ease of use and objective interpretation. However, EIA tests have substantially reduced sensitivities compared with the reference standards, and some tests only assay for one of the two toxins¹⁴. More recently, PCR-based testing methods have been used as stand-alone tests. However, since these tests identify the presence of the toxin genes (rather than the toxins themselves or their mRNA transcripts), they are often felt to be overly sensitive and have reduced specificity¹⁴.

The included studies represented a broad spectrum of patients of varying age groups and underlying oncologic diagnoses, as well as those who had previously experienced CDI. Burgner et al. reported patients of a younger age were more likely to be colonized with *C. difficile* given the mean age among their colonized patients differed significantly from their non-colonized patients (29.6 months vs. 66.7 months; $p < 0.02$)⁵¹. However, 4 of the asymptomatic patients were less than 1 year old and may have skewed these results. Neither Oskarsdóttir et al. nor Armin et al. (whose study included the largest cohort of patients) identified a relationship between age and colonization status^{62,67}, though the authors did not clearly report if children less than 1 year of age were included. Conflicting results were also seen in terms of healthcare-related exposures and *C. difficile* colonization. Dominguez et al. noted a non-statistically significant relationship between colonization and increased number of recent independent outpatient visits³⁶, whereas Burgner et al. found colonized patients had on average shorter

hospital stays (3.4 days vs. 8.3 days; $p < 0.05$)⁵¹. With regards to antibiotic exposure, Dominguez et al. reported a non-statistically significant possible relationship between colonization and increased recent antibiotic exposure³⁶, whereas Armin et al. and Oskarsdóttir et al. did not note a trend^{62,67}. These discrepancies are likely related to the small cohort sizes in each study (ranging from 45³⁶ to 152⁶²), different measures for quantifying antibiotic exposure, as well as the different timepoints when patients were tested for *C. difficile* colonization. In contrast, studies among adult populations (though not focused on oncology) have identified both recent hospitalization and exposure to certain antibiotics as significant risk factors for becoming colonized³. Conclusions regarding predictors for *C. difficile* colonization among pediatric oncology patients could not be drawn from the small number of included studies.

Chiesa et al were the only authors to test pediatric oncology patients on a repeated basis in order to follow the natural history of *C. difficile* colonization⁶⁴. Their cohort was the smallest of the included studies (N=15), and only 4 patients (26.7%) were colonized with potentially toxigenic strains of *C. difficile* (all isolates were able to produce toxin *in vitro*, however three isolates had negative cytotoxicity assays when the stool was directly tested). Patients were followed over a 12-week period, with swabs obtained weekly in both the outpatient and inpatient settings. The 3 patients with non-toxigenic *C. difficile* colonization (based on cytotoxicity assay using direct stool testing) were colonized on only one instance, whereas the patient with toxigenic *C. difficile* colonization *in vivo* was colonized consistently for one month. None of these patients developed CDI. Recent adult literature suggests patients colonized with toxigenic strains at the time of admission have an almost 6-fold higher risk of progressing to CDI compared to non-colonized patients^{3,44}. The majority of these adult studies were conducted after the identification of hypervirulent *C. difficile* strains in 1994, the cohort sizes were larger (ranging from 150 to over

3000 patients), and the follow-up duration was variable as patients were followed up to 60 days following hospital discharge³. Chiesa et al. conducted their study in the 1980s, and the conflicting results compared to the current adult literature is likely related to the small cohort size, rare outcome event (none of the colonized or non-colonized patients developed CDI), and different predominant *C. difficile* strains at the time.

The small number of included studies and small study sizes limit the generalizability of the knowledge acquired from our scoping review. In comparison to the six studies that fulfilled inclusion criteria for our review, five studies were excluded because they were published in a language other than English. It is unclear how the findings from these excluded studies may have impacted our results. In addition, the statistical association between certain predictors and *C. difficile* colonization were inconsistent between studies and the available data did not permit more robust statistical analysis such as pooled measures of association. Furthermore, there was a paucity of published information to allow for the development of a theoretical model outlining what leads to *C. difficile* colonization in the pediatric oncology population, and what contributes to either clearance of or progression to symptomatic infection. These limitations further justified the need to conduct our retrospective cohort study.

CONCLUSIONS

This scoping review summarizes the current knowledge pertaining to *C. difficile* colonization among pediatric oncology patients and identified gaps in the scientific literature. The prevalence of asymptomatic colonization ranged from less than 10% to almost 40% among different studies, with toxigenic strains accounting for the majority of identified strains. Laboratory testing

methods varied across the different studies, as did the timing of testing. There were no epidemiological or clinical features consistently identified as risk factors for colonization. Currently published literature does not adequately address the natural history of *C. difficile* colonization in this population, in terms of who is at greatest risk for developing colonization, duration of colonization, or progression to CDI.

TABLE 1 – Data Abstraction Form

Paper ID (Author, Year)	Publication Status	Study Design	Country of Origin	Aims/Purposes	Population (# patients, age, oncologic diagnoses)	Methods	Intervention & Comparator (where applicable)	Outcomes	Key Findings that relate to Scoping Review Questions (burden, risk factors, natural history)
Example 1									

TABLE 2 – Studies investigating prevalence rates of *C. difficile* colonization

Study Authors (reference)	Country, Study Duration, (Year(s) conducted)	Study Design	Setting & Patients	Timing of Testing	No. of included patients	<i>C. difficile</i> detection	Toxin detection	Prevalence (no. of positive patients/total no. of patients [%])		Additional Results
								Overall <i>C. difficile</i> colonization N (%)	Toxigenic <i>C. difficile</i> colonization N (%)	
Armin et al. ⁶²	Iran, 19 months (2008-2009)	Prospective Cohort	Oncology patients admitted to hematologic ward in a children's hospital	Admission (within 48h)	152	Culture	CPE and ELISA (toxins A & B)	38/152 (25%)	36/38 (94.7%) (by CPE) 1/38 (by ELISA)	No association between colonization or CPE and age, gender or overall antibiotic use
Brunetto et al. ⁶³	UK, 5 months (1986)	Retrospective Cohort	Patients admitted to the pediatric oncology unit	Admission (timing not specified)	63	Culture	Not clearly described	5/63 (7.9%)	Not reported	5 Asymptomatic patients had <i>C. difficile</i> isolated on 11 occasions, where toxin was detected in 2 isolations
Burgner et al. ⁵¹	Australia, 9 months (1995-1996)	Prospective Cohort	Asymptomatic pediatric oncology patients admitted to hospital	Prevalence survey repeated 12 times over 9-month period	44	Culture	ELFA (Toxin A) CPE (Toxin B)	17/44 (38.6%)	11/17 (64.7%)	Toxigenic strain carriage associated with younger age and shorter hospital stay
Chiesa et al. ⁶⁴	Italy, 12 months (1982-1983)	Prospective Cohort	Children with ALL on maintenance chemotherapy see in a pediatric oncology clinic	12-week follow-up period	15	Culture	Cytotoxicity Assay	4/15 (26.7%)	1/4 (25%)	All isolates produced toxin <i>in vitro</i> . Patient with toxigenic strain (<i>in vivo</i>) was colonized for 1 month (the rest were no longer colonized the following week)

TABLE 2 – Studies investigating prevalence rates of *C. difficile* colonization (cont'd)

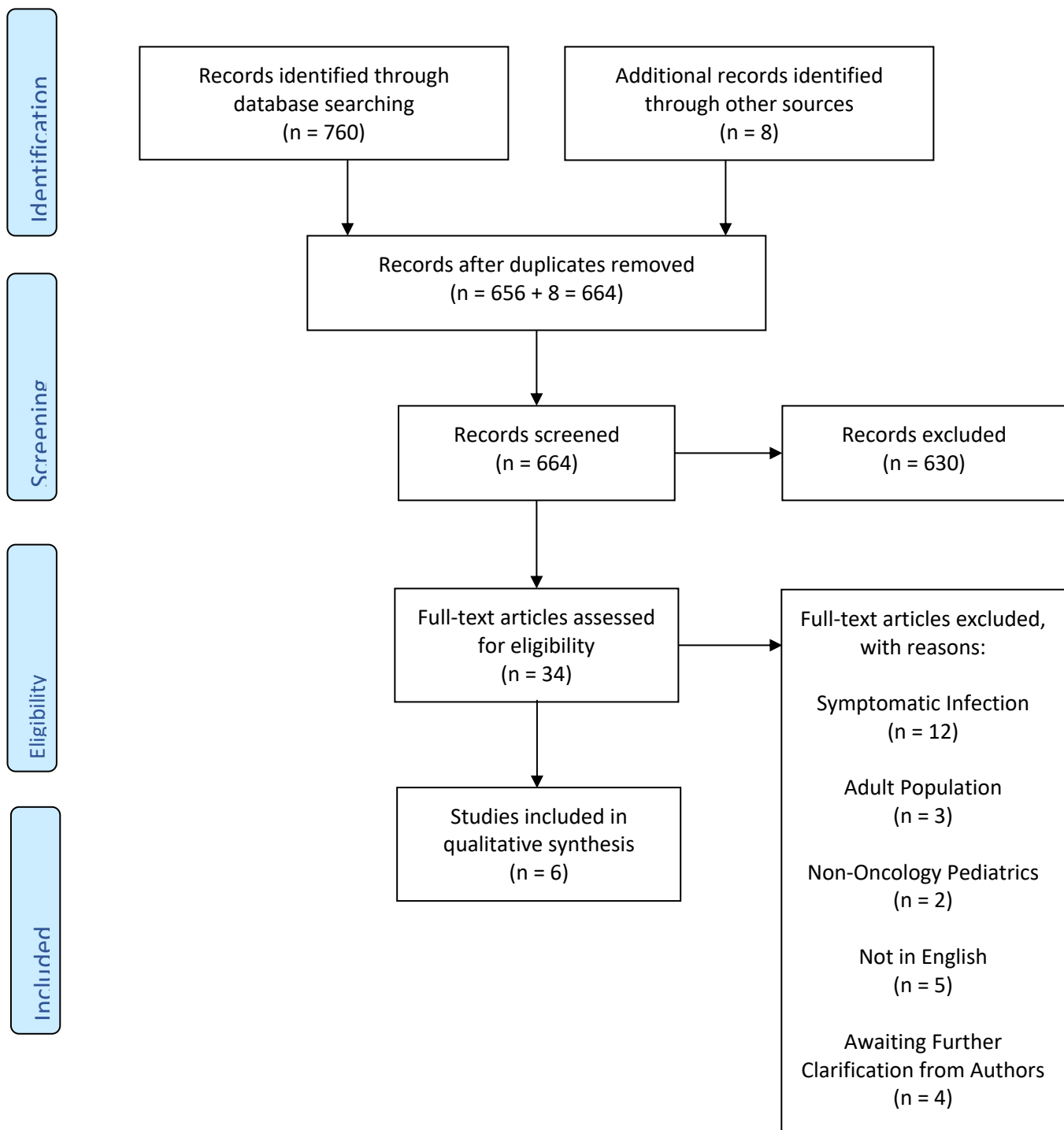
Study Authors (reference)	Country, Study Duration, (Year(s) conducted)	Study Design	Setting & Patients	Timing of Testing	No. of included patients	<i>C. difficile</i> detection	Toxin detection	Prevalence (no. of positive patients/total no. of patients [%])		Additional Results
								Overall <i>C. difficile</i> colonization N (%)	Toxigenic <i>C. difficile</i> colonization N (%)	
Dominguez et al. ³⁶	USA, 2 months (2012)	Prospective Cohort	Patients with no prior history of CDI admitted to the oncology unit at a children's hospital	Admission (within 72hr)	45	PCR	PCR (Toxin B gene)	10/45 (22.2%)	10/10 (100%)	Colonized patients had overall greater antibiotic exposure and hospital encounters in previous 12 weeks, but not statistically significant
Oskarsdóttir et al. ⁶⁷	Sweden, 19 months (1988-1989)	Prospective Cohort	Asymptomatic pediatric patients during first week on oncology ward	Admission (within first week)	46	Culture	Cytotoxin Assay	11/46 (23.9%) (positive cultures and/or toxin)	2/11 (18.2%)	Colonization at admission not related to age or previous receipt of antibiotics

FIGURE 1 – Search Strategy

Population (1a)	Population (1b)	Concept (2)	Context (3)
Paediatric Pediatric Child Adolescent Infant	Oncology Cancer Neoplasm Leukemia lymphoma	<i>Clostridium difficile</i> <i>C. difficile</i> C. diff Cdiff	Colonization Carrier state Carrier Asymptomatic
OR			
OR			
OR			
OR			

Strategy: [1a] AND [1b] AND [2] AND [3]

FIGURE 2 – PRISMA Flow Diagram



PART III: RETROSPECTIVE COHORT STUDY

INTRODUCTION

Current literature indicates in addition to higher rates of CDI among children hospitalized with cancer compared to those without¹⁶, there are substantially higher *C. difficile* colonization rates in the paediatric oncology population. In contrast to the adult literature, it is unclear which epidemiological or clinical features are predictors of colonization at the time of hospital admission among this group of patients. A scoping review revealed the literature does not adequately address the natural history of *C. difficile* colonization in paediatric oncology population, and the small number of included studies and small study sizes limit the generalizability of the findings. Therefore, a longitudinal cohort study was undertaken in order to determine if *C. difficile* colonization in the pediatric oncology population is a risk factor for subsequent symptomatic CDI and to identify predictors associated with colonization at the time of admission.

OBJECTIVES

Primary Objective

To determine if *C. difficile* colonization is a risk factor for subsequent symptomatic CDI in the pediatric oncology population.

Secondary Objective

To identify risk factors associated with *C. difficile* colonization at the time of admission among hospitalized pediatric oncology patients.

INVESTIGATIONAL PLAN

Summary of Study Design

This is a retrospective cohort study of pediatric oncology patients admitted to the oncology ward at McMaster Children's Hospital (MCH) over an 18-month period. Patients who were routinely screened for antibiotic-resistant organisms (AROs) upon admission per hospital policies had their stored samples subsequently tested for *C. difficile*. Among patients whose samples were tested for *C. difficile*, a retrospective chart review was completed to determine if any of these patients subsequently developed CDI. The retrospective analysis was also conducted to determine risk factors for asymptomatic colonization.

Study Design Considerations

Different study designs were considered in order to answer the study objectives. They are discussed below.

Retrospective Cohort Study

In a cohort study, a group of patients is identified based on their exposure status and then followed over the study period to determine if they develop the outcome of interest⁶⁸. When conducted retrospectively, patients have already developed the outcome and data that were collected in the past are then analyzed to identify the exposure status of patients. Since the exposure is always known to occur before the outcome, cohort studies have a temporal framework to assess causality and the potential to provide strong scientific evidence. Furthermore, multiple exposures and outcomes can be studied simultaneously. Compared to other observational study methods, cohort studies allow for the calculation of incidence rates in addition to prevalence rates. Retrospective cohort studies tend to be less costly and require less time to complete due to the immediate

availability of data. If the data can be obtained without patient interviews, these studies can also be free from recall bias. However, the investigator has limited control over the data collection process and the data may be incomplete, inaccurate or inconsistently measured^{68,69}.

Given this project has multiple objectives where the outcomes and exposures differ, a cohort study design would be preferred over a case-control study. Using a retrospective design combined with running additional PCR testing for presence of *C. difficile* on existing, frozen swabs that had been collected as part of routine care (i.e. MRSA/VRE screening of admitted patients) would obviate the need for the collection of additional swabs or contact with the study team. The swabs were readily available therefore facilitating the completion of this project within the timeframe of a Master's thesis. In addition, variables such as *C. difficile* colonization, antibiotic exposure, healthcare exposure could all be collected objectively and limit recall bias. Hence, the retrospective cohort design was the most appropriate for this study.

Prospective Cohort Study

In contrast, when conducting a prospective cohort study a group of patients defined by their exposure status are followed forward in time to determine those who develop the outcome of interest. In addition to the general advantages of the cohort design listed above, prospective cohort studies allow the investigator to tailor the methods for collecting specific exposure data thus potentially providing a more complete data set. However, it may take time to screen many patients to identify those who are exposed if the exposure rate is rare. Furthermore, there may be a lengthy follow-up period if the outcome of interest has a long latency period and subjects may be lost to

follow-up over time. Both of these disadvantages can dramatically increase the cost of conducting a prospective cohort study.^{68,69}

If this project were designed prospectively, patients would have swabs obtained per routine ARO-screening hospital policies and *C. difficile* PCR testing performed in the laboratory as the swabs arrive for MRSA and VRE testing. As a result, *C. difficile* testing may be more complete as samples would not need to be retrieved from storage. The collection of certain variables, for example the development of diarrheal symptoms, would also be more complete as patients would be assessed daily. However, it would not be possible to quickly abstract all the data for all enrolled patients as the data collection would need to progress on a day-by-day basis. This lack of efficiency is the main reason for not pursuing a prospective design for the purposes of a this Master's thesis.

Nested Case-Control Study

In a case-control study, subjects are identified based on their outcome status at the outset of the study. Using the nested case-control design, after cases are identified, a pre-specified number of controls is selected from the same cohort who have not yet developed the disease by the time of disease occurrence in the case. Therefore, time-matching is an essential feature of this design. In addition, since case-control studies do not follow subjects through time, a cohort member who serves as a control at one point in time may later become a case. The nested case-control design is more efficient than a cohort study, as the exposure of interest need only be measured among cases and selected controls. Therefore this study design would be chosen in scenarios where the outcome is rare in addition to the exposure of interest being difficult or expensive to obtain⁷⁰.

For the study's primary objective, cases would be patients who develop CDI during the study period and controls would be identified from the same cohort as those without disease at the time when a case of CDI is diagnosed (i.e. the cases and controls would be time-matched). The exposure of interest would be *C. difficile* colonization status. For the secondary objective, a second set of cases and controls would be defined by *C. difficile* colonization status upon admission (where cases and controls would be matched by admission date +/- 3 days), and the exposures of interest would be various potential epidemiological and clinical risk factors. However, for both study objectives, the disease status of interest (i.e. CDI or *C. difficile* colonization) are not rare occurrences based on existing literature. As a result, most of the cohort would likely need to be analyzed therefore negating the gains in efficiency while sacrificing the ability of using the information collected on the full cohort.

Case-Crossover Study

A case-crossover study is a type of “within subject” study design, where an individual acts as their own case and control. Cases are defined and then assessed for their exposure status immediately prior to the time when they became a case and compared to their exposure at a prior timepoint where they were not a case (i.e. they were a control). Since an individual acts as their own control, fewer subjects are needed and this design can minimize bias due to variability between participants. These types of studies are particularly useful in establishing exposure-outcome relationships where the outcome is acute and well defined. However, the reliance upon prior exposure time requires that the exposure does not have an additive or cumulative effect over time. Depending on how exposure history is collected, there is the potential for higher recall bias compared to other study designs ^{68,71}.

In this study, each patient will have repeated measurements (i.e. at the time of each hospital admission) therefore creating numerous timepoints when each patient could be either a case or control. Similar to using a nested case-control design, a separate set of cases and controls would need to be defined for each study objective. Given this study will take place at a single site over a limited time span, a case-crossover design would be an efficient method that would not require a large number of subjects to be enrolled. However, epidemiological exposures of interest such as hospitalization and antibiotic administration have an additive effect over time thus unfortunately limiting the utility of this study design.

In summary, the retrospective cohort study design was chosen as the most appropriate for our research question.

Study Duration and Enrollment

Patients were considered for study inclusion if they were admitted to the oncology ward at MCH between September 1, 2016 and February 28, 2018. Follow-up information through May 31, 2018 was collected. Based on previous pilot work, it was expected that swabs and clinical data would be collected from 250 admissions during this study timeframe.

Study Population

The study included paediatric oncology patients admitted to the oncology ward at MCH who had at least one rectal swab obtained for ARO screening during the study period.

Inclusion Criteria

A patient was included if they fulfilled all of the following criteria: age < 18 years, admitted to the pediatric oncology ward, ARO rectal screening swab collected within 72hr of hospital admission, absence of diarrhea at the time of ARO screening.

Exclusion Criteria

Infants <1 year were excluded because they have higher colonization rates³⁴.

STUDY PROCEDURES

Antibiotic-Resistant Organism (ARO) Screening

Per MCH infection control policies, patients who have been admitted to any hospital within the prior year are routinely screened upon admission for MRSA and VRE using rectal swabs collected within 72 hours of admission. Many important risk factors for colonization or infection with these AROs are universal, including previous exposure to healthcare settings and prior antibiotic exposure, specifically antibiotic misuse⁷². Among hospitalized pediatric patients, those with oncologic diagnoses are admitted to hospital on a repeated basis to receive chemotherapy and for management of complications from their immunosuppressive therapy such as mucositis, chemotherapy-induced nausea and vomiting, and infections. Furthermore, as many of these patients will experience infectious complications, this group of patients also experiences a significant burden of antibiotic exposure, a known risk factor for CDI. Therefore, the current hospital policy for ARO screening appropriately identifies oncology patients as a group at risk for CDI or *C. difficile* carriage in addition to MRSA and VRE carriage^{15,44,72}.

Collection of swabs and testing for *C. difficile*

Upon admission to the oncology ward, nurses screen patients to identify those who require ARO screening. The nurse primarily caring for the admitted patient will swab the anterior nares and rectum using eSwabs™ (Copan Italia, Brescia, Italy), and submit the swabs to the laboratory in Amies transport medium as per routine practice. Upon accessioning at the microbiology laboratory, rectal swabs are plated and tested for the presence of MRSA and VRE. Subsequently, they are routinely frozen in case they are required for future testing. Stool in the eSwab™ medium is sampled without extraction, therefore the sample does not need further processing prior to storage. Swabs that were collected during the study period were marked for future *C. difficile* testing.

In this study, *C. difficile* carriage was determined using a validated, laboratory-developed molecular testing platform for *C. difficile*, the loop-mediated isothermal amplification (LAMP) test. This is a quantitative PCR assay that identifies the *tcd* gene (the regulatory gene for the *C. difficile* toxin gene) and in turn directly detects the presence of toxigenic *C. difficile* ⁷³ and has been shown to be 98% sensitive and specific as compared to accepted combination reference methods (cytotoxin B assay and toxigenic culture) for *C. difficile* testing ⁷⁴. This assay is routinely used in the clinical laboratory for detection of *C. difficile* toxin genes when symptomatic patients have diarrheal stools tested for *C. difficile*. Recent studies have demonstrated the utility of using perirectal and rectal swab samples to detect asymptomatic carriage by molecular PCR methods ^{75,76}, therefore the screening rectal swabs for MRSA and VRE detection were judged to be adequate when testing for *C. difficile* carriage.

Once enrollment was complete, the specimens were thawed for further testing. They were briefly heated to 95 degrees Celsius and then amplified using the laboratory-developed LAMP. This process involved isothermal amplification at 60 degrees Celsius for 50 minutes, and then the DNA (deoxyribonucleic acid) product was detected using calcein on the QIAGEN RotorGene. A specimen was considered positive if the amount of detected genetic material crossed the threshold in less than 45 minutes, and indeterminate if it crossed the threshold in 45-50 minutes. Positive and indeterminate results were extracted using easyMAG extraction (bioMerieux, City, state), and confirmed using PCR methods (to detect toxin A gene) as part of the laboratory-based validation process.

Definition of *C. difficile* colonization and CDI Case Definition

Our case definition for CDI was guided by the Provincial Infectious Diseases Advisory Committee (PIDAC) of Public Health Ontario, the Canadian Nosocomial Infection Surveillance Program (CNISP) and the Canadian Paediatric Society (CPS)^{31,77,78}:

1. diarrhea (3 or more loose/watery stools in a 24-hour period that are unusual or different for the patient, without a recognized etiology for the diarrhea), or fever, abdominal pain and/or ileus AND a laboratory confirmation of a positive PCR for toxigenic *C. difficile* (LAMP assay)

OR

2. visualization of pseudomembranes on sigmoidoscopy or colonoscopy (or after colectomy)

OR

3. histological/pathological diagnosis of pseudomembranous colitis

OR

4. diagnosis of toxic megacolon

Patients who had a positive *C. difficile* test but do not meet criteria for CDI were deemed colonized.

Data Sources

Retrospective chart review

All patients meeting the inclusion criteria had their electronic health records reviewed by a single reviewer using a standardized data collection form. In addition, for all patients who had clinical samples (i.e. diarrheal stools) testing positive for *C. difficile* carriage, we determined if they had symptoms fulfilling the criteria for CDI.

Infection Prevention and Control (IPAC) surveillance data

IPAC practitioners routinely review patients with diarrhea. Among those who test positive for *C. difficile*, they prospectively determine with their medical lead whether the patients meet the CDI case definition. The IPAC surveillance data obtained during the study period was also reviewed to ensure complete identification of all CDI cases among oncology inpatients during the study period. Any discrepancies between the IPAC surveillance data and the retrospective chart review while identifying CDI cases were discussed with the thesis supervisor.

Abstracted Data Elements

Patient electronic medical charts were accessed to obtain demographic information, details regarding their cancer diagnosis and treatment, antimicrobial use during previous and current hospital admissions, other relevant medication exposures, episodes of diarrhea and other symptoms concerning for CDI, previously documented CDI, and previously isolated AROs. The

microbiology laboratory provided *C. difficile* testing results from stored ARO screening samples. Lastly IPAC CDI surveillance data was also utilized. (See *Appendix 1*)

An Antibiotic-Risk Score was calculated to capture inpatient antibiotic use during the previous 3 months. It was based on the days of therapy when patient received antibiotics associated with increased CDI risk (i.e. 3rd generation cephalosporins, beta-lactam-beta-lactamase inhibitor combinations, carbapenems, fluoroquinolones, clindamycin)⁷⁹⁻⁸¹. As patients rarely receive more than one of these classes of medication simultaneously, the days of therapy was calculated regardless if they were receiving one or more antibiotic.

STATISTICAL CONSIDERATIONS

Sample Size and Power

Data from a previous pilot study of high-risk inpatients revealed 8.3% of 300 anonymously-tested MRSA swabs were positive for *C. difficile*. From historical data, we anticipated 280 admission to the paediatric oncology ward over a 1-year period. We estimated 80-90% of admissions to meet ARO screening criteria. Thus, we expected roughly 250 samples yielding 20 positive results over the duration of the study. The initial study period was 12-months, however was increased to 18-months to allow for the collection of 250 samples to facilitate univariable and some limited multi-variable logistic regression analysis.

Baseline Characteristics

Standard descriptive statistics were used to examine the study sample's baseline and demographic characteristics. Continuous variables were reported using means and standard deviations for normally distributed data, or as median and quartiles (quartile 1, quartile 3) for skewed data.

Number and percentage were reported for categorical variables. The student's t-test (for normally distributed data) or the Mann-Whitney U test (for non-normally distributed data) was used to compare continuous variables, and the χ^2 test or Fisher's exact test was used to compare categorical variables between groups (colonized vs. never colonized) as appropriate.

Primary analysis

We conducted a logistic regression to examine if colonization status was a predictor of the primary dichotomous endpoint, CDI. Relevant co-variables (age, gender, type of malignancy, current use of enteral feeds, current use of stomach-acid reducing agents, current therapy with broad-spectrum antibiotics, number of hospital-admission days in preceding 6 months, and previous antibiotic exposure in the last 3 months) were initially evaluated using univariable logistic regression. Variables with a p-value < 0.05 were considered for inclusion in the multivariable model. The final model was limited to 2 or 3 variables and determined using a step-wise forward selection method.

Secondary Analysis

We conducted univariable logistic regression to examine which risk factors were predictors of the dichotomous endpoint, *C. difficile* colonization. The *a priori* hypothesis was that subjects with underlying gastrointestinal comorbidity, hematologic malignancy, use of enteral feeding, use of high-dose corticosteroids in the last 3 months, use of stomach-acid suppression in the last 3 months, the number of days spent in hospital during the previous 6 months and previous burden of broad-spectrum antibiotic exposure were more likely to develop *C. difficile* colonization. Variables with a p-value < 0.05 were considered for inclusion in the logistic regression model.

First, a limited multi-variable logistic regression analysis was conducted with the final model limited to 2 or 3 variables and determined using a step-wise forward selection method. Goodness of fit was determined using the Hosmer-Lemeshow and Chi-Square goodness of fit tests.

Second, the correlation between the selected patient characteristics and colonization status was also tested using repeated measures logistic regression. The multiple observations taken from each patient (i.e. at each admission) are likely correlated and would violate the assumption of independence between observations to avoid erroneous inferences from a simple logistic regression (SLR), the generalized estimating equations (GEE) can be used in order to utilize the data available from all available observations. Given the dichotomous dependent variable, a GEE assuming a binomial probability distribution and logit link function were chosen for the model. The Corrected Quasi Likelihood under Independence Model Criterion (QICC) was used as the goodness-of-fit measure, enabling one to choose between two sets of model terms, given a correlation structure. It assumes that the distribution, link function and working correlation matrix specifications are all correct for the dataset. The model that obtains the smaller QICC is better according to this criterion.

We expected that patients with hematologic malignancies were more likely to have received high-dose corticosteroids (as this is common component of the chemotherapy regimens for hematologic malignancies), and spent more time admitted to hospital and have greater exposure to broad-spectrum antibiotics (due to the increased immunosuppressive effects of the hematologic malignancy chemotherapy regimens) than patients with solid organ malignancies. Therefore, multicollinearity will be assessed using model tolerance and standardized errors of the

beta-coefficients. In addition, interaction terms that combined malignancy type and each of these other variables were created and tested for significance using the likelihood ratio test. Significant terms were added to the final multivariable model.

Statistical analyses were performed using SPSS Statistics for Macintosh, version 25.0 software (IBM, Armonk, NY). *P* values < .05 were considered statistically significant.

RESULTS

From September 1, 2016 through February 28, 2018, 338 ARO screening swabs were collected and tested using the *C. difficile* LAMP assay. After excluding 45 swabs that were obtained beyond 72 hours after admission, 10 swabs collected when patients were admitted to other areas of the hospital and 24 from patients without underlying oncologic diagnoses, 259 remained for analysis.

Patient Characteristics

Swabs from 259 admissions representing 74 unique patients were included, as shown in Figure 1. The median number of admissions per patient over the study period was 5 (3, 9), with ARO screening swabs obtained and tested for *C. difficile* colonization on 48.2% of admissions. Each patient had screening swabs tested from a median of 2 hospital admissions (1, 4). In total, there were 279 admissions that did not have *C. difficile* colonization results available, including 116 admissions where swabs were not obtained and 159 admissions where swabs were not tested because they were not received by the research laboratory. The median time between the first and last admission swabs during the study period was 1.9 months (0.2, 4.8). The duration of follow-up for CDI ranged from 3 months to 20 months. Two patients were lost to follow-up; one

patient died on May 15 2017 and another did not have any further visits with their oncologist after September 13 2017.

The median age at the time of study entry was 6.9 years (3.7, 11.7). Over half of the patients were male (n=46, 60.0%). As expected in this pediatric oncology population, hematologic malignancies were more common than solid tumors (n=45 (60.8%) vs. n=29 (39.2%)). Three patients were transplant recipients, including 1 with a solid organ transplant and 2 with bone marrow transplants. About one third of patients (n=23, 31.3%) had other underlying medical conditions. The three most commonly reported types included neurologic/psychiatric (n=7), respiratory (n=5), benign blood disorders (n=3) and genito-urinary (n=3). Nine patients (12.2%) had a previous episode of CDI prior to their first swab collected as part of this study (See *Tables 1 and 2*).

The comparison of baseline characteristics between the 9 patients colonized with *C. difficile* upon at least one admission and the 65 patients who were not colonized did not yield any statistically significant differences. (See *Table 1*)

***C. difficile* Colonization**

Over the course of the study, 9 patients (12.2%) were found to be colonized on at least one admission. Seven of these patients (77.8%) were found to be colonized on only one admission, and two (22.2%) were colonized on three instances. Duration of colonization ranged from less than 7 days (i.e. only one swab was positive with the closest follow-up swab a week later) to 55 days (obtained from a patient with positive swabs on three consecutive admissions). Of the 9 patients who were colonized, only 2 (22.2%) had a preceding CDI prior to their first positive

swab. One of these patients had a negative swab following their CDI episode, and one had a positive swab 30 days after their CDI episode. (See *Figure 2*)

Progression to CDI

The 9 patients who were colonized during the study were monitored until May 31 2018 for the development of CDI. The median follow-up duration was 17.9 months (15.0, 19.0). None of these patients went on to develop CDI during the study period. Therefore, a regression analysis to determine risk factors for progression to CDI was not possible.

Risk Factors for *C. difficile* Colonization – Overall Study Cohort

Predictors of *C. difficile* colonization are outlined in Table 3. When colonized patients (n=9, 12.2%) were compared to those who were never colonized (n=65, 87.8%), age at baseline, sex, presence of other comorbidities, type of malignancy (hematologic vs. solid tumor), or previous CDI were not associated with colonization. (See *Table 3*)

A limited multivariable analysis was done given there were only 9 colonized patients in the cohort. After adjusting for age at baseline and sex, previous CDI did not predict colonization. After the addition of controlling for the receipt of enteral feeds, previous CDI still did not predict colonization. (See *Table 4*)

Assumption Testing and Model Fit

Given the dichotomous dependent variable used for this analysis, a logistic regression approach to modeling was chosen. The Hosmer-Lemeshow (HL) test was used as the primary goodness-

of-fit measure, providing information on the degree of superiority in fit of the current model to the null model without covariates.

From the analysis, the final model with 4 predictors (age, sex, previous CDI and enteral feeds) represented a good fit under the null hypothesis of good fit (HL χ^2 (df 8) of 5.289, $p=.726$).

Another model, which excluded enteral feeds, also had a good fit (HL χ^2 (df 8) of 4.123, $p=.846$).

Neither model reached statistical significance under the Omnibus Test. (See *Table 4*)

Risk Factors for *C. difficile* Colonization – Colonized Patients

Eight (88.9%) of the colonized patients had positive and negative screening swabs obtained during the study period. GEE with a logit link was chosen to determine risk factors for this dichotomous outcome, allowing for the analysis of repeated measures in a case-crossover setup. These patients had swabs collected from fifty-one admissions (19.7% of all admissions included in analysis) that contributed data to this analysis. The median number of admissions per patient was 5 (4, 6). Predictors of colonization are outlined in Table 5. In univariable analyses, malignancy type (hematologic vs. solid tumor) was significantly correlated with *C. difficile* colonization (OR = 3.64; 95% CI (1.32, 9.98); $p = 0.012$). However, age, sex, presence of other medical comorbidities, previous CDI, neutropenia, use of stomach acid suppressant medication, enteral feeds, number of days admitted to hospital (either over the past 3 months or 6 months) or recent antibiotic exposure were not associated with colonization. (See *Table 5*)

A limited multivariable analysis was done given there were only 12 admission swabs that were positive. After adjusting for age, type of malignancy and previous CDI, neither recent hospitalization in the last 6 months nor high-risk antibiotic exposure during the previous 3

months was significantly correlated with the outcome. After creating a 5-variable model containing all of these factors, both, recent hospitalization during the last 6 months and high-risk antibiotic exposure during the previous 3 months remained uncorrelated with *C. difficile* colonization. (See *Table 6*)

Assumption Testing and Model Fit

From the analysis, the model with 4 variables (including age, type of malignancy, previous CDI and hospitalization during the previous 6 months) had a QICC = 60.152, whereas the model with 4 variables (including age, type of malignancy, previous CDI and high-risk antibiotic exposure during the previous 3 months) had a QICC = 61.726. A third model, which included 5 variables, generated a QICC of 62.053. When comparing these three models, the lowest QICC was observed from the model with 4 variables (including hospitalization in the last 6 months) indicating a better fit. An independent working correlation matrix was chosen because it provided the best model fit. (See *Table 6*)

TABLE 1 – Baseline Characteristics of Children Admitted to the Oncology Ward Who Had *C. difficile* Colonization Tested at Admission

	Overall N=74 (%)	Colonized N=9 (%)	Never Colonized N=65 (%)	<i>p</i> -value* (Comparing Colonized vs. Never Colonized)
Age in years at first swab (median; quartile 1, quartile 3)	6.9 (3.7, 11.7)	10.7 (5.1,14.5)	6.6 (3.5, 11.0)	0.172
Male patients	46 (59.0%)	7 (77.8%)	36 (55.4%)	0.288
No Other Medical Conditions	51 (68.9%)	6 (66.7%)	45 (69.2%)	<0.999
Other Comorbidities				
- Neurologic/Psychiatric	7	1	6	
- ENT	1	0	1	
- Respiratory	5	0	5	
- Cardiovascular	2	1	1	
- Gastrointestinal	1	1	0	
- Endocrine	2	0	2	
- Hematologic	3	0	3	
- Genitourinary	3	1	2	
- Previous Cancer	1	0	1	
- Complex Care (> 3 systems involved)				
Malignancy				
- Hematologic	45 (60.8%)	6 (66.7%)	39 (60.0%)	<0.999
- Solid organ	29 (39.2%)	3 (33.3%)	26 (40.0%)	
Transplant	3 (4.1%)	0 (0%)	3 (4.6%)	<0.999
- Solid Organ Transplant	1		1	
- Bone Marrow Transplant	2		2	
Previous CDI	9 (12.2%)	2 (22.2%)	7 (10.8%)	0.300

* *p*-values reported for student's t-test, Mann-Whitney U test, χ^2 test or Fisher's exact test as appropriate

TABLE 2 – Primary Oncologic Diagnoses

Cancer Diagnoses	Overall Cohort N=74 (%)
Hematologic	
Acute Lymphoblastic Leukemia (ALL)	29 (39.2)
Acute Myeloid Leukemia (AML)	1 (1.4%)
Hodgkin's lymphoma	10 (13.5%)
Other lymphoma	4 (5.4%)
Solid Tumors	
Brain Tumors	
Neuroblastoma	9 (12.2%)
Medulloblastoma	4 (5.4%)
Ependymoma	2 (2.7%)
Other brain tumors	3 (4.1%)
Osteosarcoma	3 (4.1%)
Rhabdomyosarcoma	3 (4.1%)
Wilms Tumor	2 (2.7%)
Other	4 (5.4%)

TABLE 3 – Predictors of *C. Difficile* Colonization (All Patients, N = 74)

	Univariable Analysis		
	OR	95% CI	<i>p</i> -value
Age at Baseline	1.08	0.95, 1.24	0.230
Male Sex	2.82	0.54, 14.62	0.217
Any other comorbidity	1.12	0.25, 4.95	0.876
Hematologic Malignancy Compared to Solid Tumor	0.75	0.17, 3.27	0.702
Previous CDI	2.37	0.41, 13.71	0.336
Enteral feeds at baseline (via NGT, OGT, GT, GJT)	0.31	0.07, 1.36	0.122

NGT nasogastric tube, OGT oral-gastric tube, GT gastrostomy tube, GJT gastro-jejunosomy tube

**TABLE 4 – Predictors of *C. difficile* Colonization (All Patients, N = 74)
 Assumption Testing and Model Fit of Multivariable Analysis**

	Multivariable Analysis (Model with 3 Predictors)			Multivariable Analysis (Model with 4 Predictors)		
	OR	95% CI	p-value	OR	95% CI	p-value
Age at Baseline	1.13	0.97, 1.32	0.109	1.14	0.96, 1.34	0.132
Male Sex	4.07	0.70, 23.55	0.12	3.84	0.61, 24.38	0.153
Previous CDI	5.83	0.72, 46.98	0.10	7.13	0.42, 121.63	0.175
Enteral feeds at baseline (NGT, OGT, GT, GJT)	-	-	-	0.16	0.02, 1.16	0.070
Hosmer-Lemeshow Test	χ^2 (df 8) of 4.123, $p=0.846$			χ^2 (df 8) of 5.289, $p=0.726$		
Omnibus Test of Model	χ^2 (df 3) of 5.946, $p=0.114$			χ^2 (df 4) of 8.402, $p=0.078$		

NGT nasogastric tube, OGT oral-gastric tube, GT gastrostomy tube, GJT gastro-jejunostomy tube

TABLE 5 – Predictors of *C. Difficile* Colonization Using GEE (Colonized Patients, N = 9)

	Univariable Analysis		
	OR	95% CI	p-value
Age (years)	1.06	0.91, 1.25	0.434
Male sex	0.45	0.03, 7.33	0.578
Any other comorbidity	1.71	0.48, 6.06	0.403
Hematologic malignancy compared to solid tumor	3.64	1.32, 9.98	0.012
Previous CDI	1.23	0.51, 2.94	0.646
Neutropenic	0.79	0.26, 2.41	0.684
Taking stomach acid reducing medication	1.18	0.42, 3.31	0.755
Antibiotic treatment	1.52	0.41, 5.62	0.527
Enteral Feeds (NGT, OGT, GT, or GJT)	1.44	0.43, 4.81	0.556
Number of hospitalized days (previous 6 months)	1.00	0.97, 1.02	0.885
Number of hospitalized days (previous 3 months)	0.99	0.96, 1.02	0.711
High-Risk Antibiotic Exposure (previous 3 months) *	0.94	0.82, 1.07	0.339

NGT nasogastric tube, OGT oral-gastric tube, GT gastrostomy tube, GJT gastro-jejunostomy tube

* calculated as DOT of the following antibiotics (3rd generation cephalosporins, beta-lactam/beta-lactamase inhibitors, carbapenems, clindamycin, fluoroquinolones) in last 3 months

**TABLE 6 – Predictors of *C. difficile* Colonization Using Repeated Measures (Colonized Patients, N = 9)
Assumption Testing and Model Fit of Multivariable Analysis**

	Multivariable Analysis (Model with 4 Variables; including Recent Antibiotic Exposure)			Multivariable Analysis (Model with 4 Variables, including recent Hospitalization in last 6 months)			Multivariable Analysis (Model with 5 Variables)		
	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value	OR	95% CI	<i>p</i> -value
Age	1.10	0.87, 1.40	0.402	1.11	0.89, 1.38	0.351	1.10	0.90, 1.34	0.340
Hematologic Malignancy	3.22	1.23, 8.45	0.018	8.14	1.55, 42.72	0.013	8.27	1.40, 49.00	0.020
Previous CDI	1.63	0.32, 8.25	0.56	0.95	0.35, 2.63	0.929	0.84	0.33, 2.10	0.703
High-Risk Antibiotic Exposure (previous 3 months) *	1.00	0.88, 1.14	0.945	-	-	-	0.97	0.81, 1.16	0.734
Number of hospitalized days (previous 6 months)	-	-	-	1.03	0.99, 1.08	0.120	1.04	0.98, 1.10	0.202
QICC (Independent Matrix)	61.726			60.152			62.053		

QICC Corrected Quasi Likelihood under Independence Model Criterion

* calculated as DOT of the following antibiotics (3rd generation cephalosporins, beta-lactam/beta-lactamase inhibitors, carbapenems, clindamycin, fluoroquinolones) in last 3 months

FIGURE 1 – Colonization Upon Admission (All Patients, N = 74)

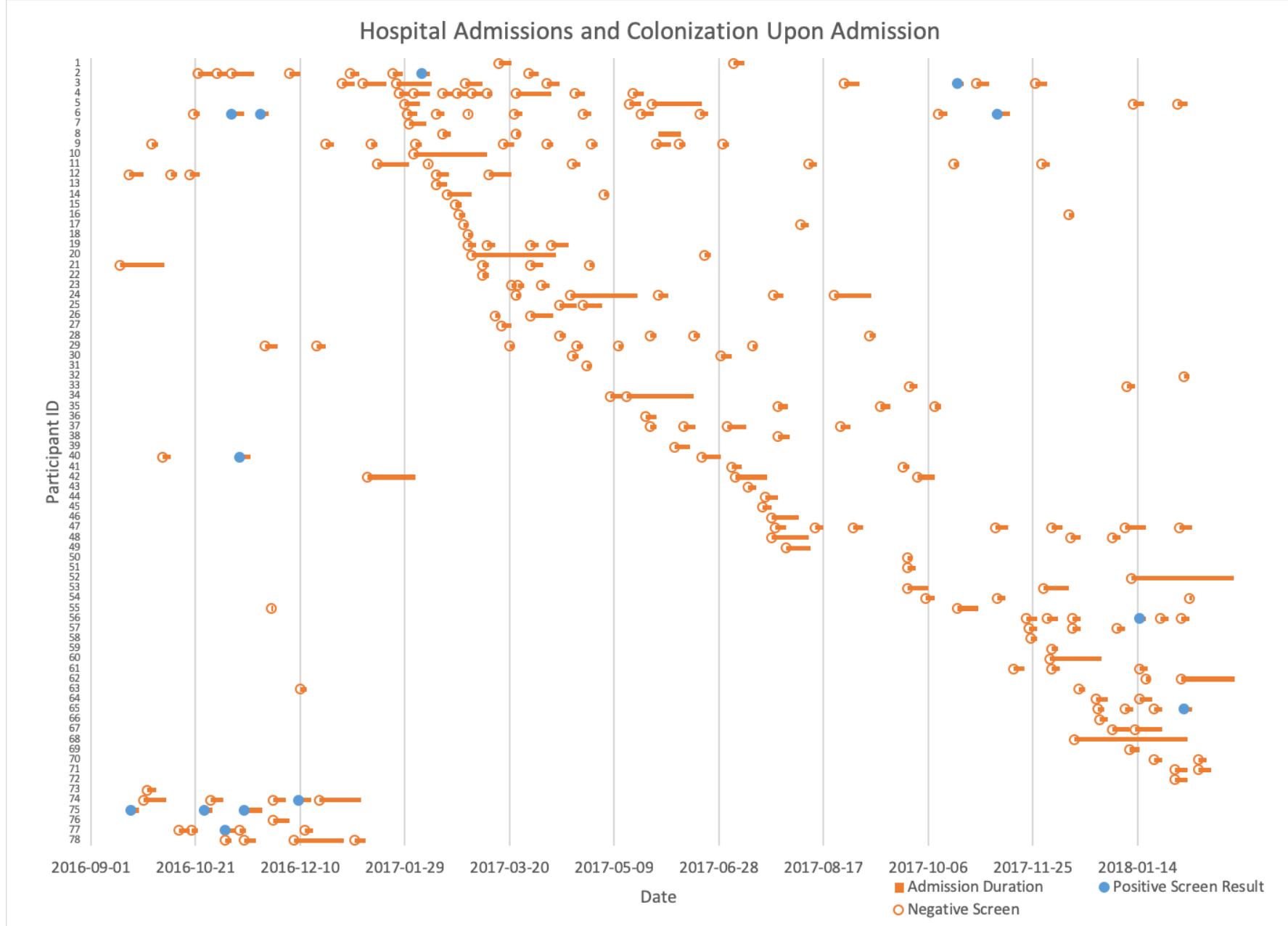
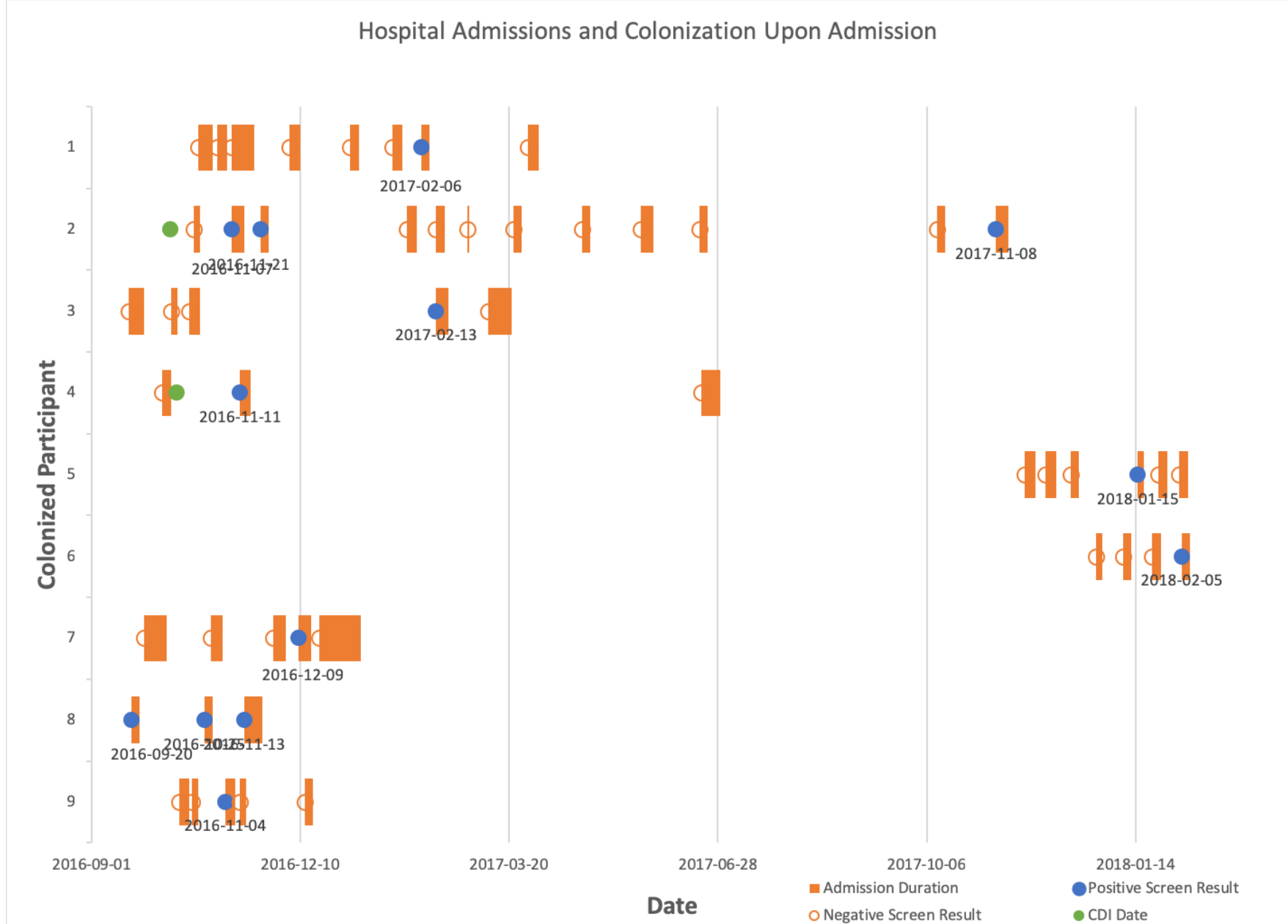


FIGURE 2 – Colonization Upon Admission (Colonized Patients Only, N = 9)



PART IV: DISCUSSION

SUMMARY OF FINDINGS

Our retrospective cohort study identified 74 patients who were admitted to the pediatric oncology ward during the 18-month period, of whom 9 developed *C. difficile* colonization, corresponding to an admission prevalence of 12.2%. Colonization was likely brief, as *C. difficile* was identified on a single admission for the majority of colonized patients. When we compared colonized patients to those who had never been colonized, we were unable to identify any baseline characteristics that were predictive of colonization. Among patients who had both positive and negative screening swabs during the study period, using a GEE for repeated measures we were unable to identify other variables that were predictive of colonization. None of the colonized patients in our cohort developed CDI. (see Figure 1)

PREVALENCE OF *C. DIFFICILE* COLONIZATION

We report on the admission prevalence of *C. difficile* colonization over an 18-month study period at a single pediatric oncology ward. Compared to the other studies identified in the scoping review, our colonization rate of 12.2% falls within the lower end of the range of reported prevalence rates. However, the meaningfulness of this comparison is unclear because prevalence rates were obtained differently. Among the studies that assessed colonization at the time of hospital admission, methods varied with regards to exact timing of obtaining patient samples, microbiological tests used to isolate *C. difficile* and identify toxigenic strains, as well as study duration. Furthermore, all of the studies used fresh stool whereas we used rectal swabs. Standardizing the definition of prevalence in this patient population with regards to these features would enable more meaningful comparison between studies.

In our study, routinely collected rectal MRSA and VRE screening swabs were used for the detection of *C. difficile* carriage. Instead of fresh stool being submitted, stool that is picked up from the rectal swab would be contained in the transport medium. The transport medium was subsequently tested for the presence of toxigenic *C. difficile* using the laboratory-developed LAMP test (a type of PCR assay). Previous studies have demonstrated the utility of using perirectal and rectal swab samples to detect asymptomatic carriage by molecular PCR methods^{75,76}. Curry et al. demonstrated a sensitivity of 100% and specificity of 99.1% when PCR methods were compared to toxigenic anaerobic culture of perirectal swabs⁷⁵, and Shakir et al. demonstrated 100% sensitivity and 100% specificity when rectal swabs were compared to stool specimens using their PCR method⁷⁶. Curry et al. employed broth preamplification of the specimens to enhance sensitivity of the PCR-based strategy and scored their swabs for visible feculence during the validation phase, neither of which was done in our study. However, the hospital policy and training of nurses emphasize the importance of obtaining fecally-stained swabs. Therefore, our screening swabs may have had a lower ability to detect *C. difficile* as compared to the sensitivity rates reported in the literature. This may explain the lower prevalence of colonization in our study as compared to other pediatric oncology studies in the scoping review, including the study by Dominguez et al. that also utilized PCR methods for detecting toxigenic *C. difficile*³⁶.

Aside from the microbiological testing considerations discussed above, there are a number of other environmental factors that could have contributed to the prevalence rate we observed. Our study was conducted on a pediatric oncology ward where the majority of patient rooms are private rooms with single occupancy. By decreasing the opportunities for patient-to-patient

contact and having nurses' patient assignments in different rooms, this would decrease the likelihood for *C. difficile* to be spread between patients either directly, or via healthcare providers. During our study period, there were no CDI outbreaks. Therefore, the circulating strains may have lacked microbiological features that would have facilitated transmission between patients. Furthermore, our study took place after a CDI outbreak had taken place on the oncology ward earlier in 2016. As a result, clinicians may have been more diligent with infection prevention practices that would have resulted in prompt isolation and testing of symptomatic patients, more frequent use of personal protective equipment and decreased transmission between staff and patients.

PATIENT CHARACTERISTICS AND *C. DIFFICILE* COLONIZATION PATTERNS

The retrospective cohort study was completed at McMaster Children's Hospital, a tertiary care pediatric centre located in Hamilton, Ontario, Canada. The division of hematology/oncology is one of the main partners in the network of POGO (the Pediatric Oncology Group of Ontario) and is a full member of COG (Children's Oncology Group), serving pediatric oncology patients from a large catchment area. As such, the 74 patients in this diverse cohort covered a range of ages and underlying oncologic diagnoses, as well as newly diagnosed and established patients. The comparison of baseline characteristics between the colonized group and never colonized group did not yield any statistically significant differences.

Seven of the nine colonized patients (77.8%) were colonized on a single admission. Six had subsequent negative swabs, that were obtained from 7 to 69 days later. Therefore, colonization was as brief as 1 week, similar to the findings by Chiesa et al⁶⁴. Among the two patients with consecutive positive swabs, colonization lasted from 14 to 55 days. It is difficult to draw

definitive conclusions about the upper limits of colonization duration for a number of reasons.

With the pragmatic approach to swab collection in this study, swabs were collected at irregular intervals based on timing of hospital admission. This could be improved in future study designs by obtaining swabs at outpatient clinic visits. Furthermore, each of these patients had admissions for which *C. difficile* colonization information was not available, ranging from 28.6% to 58.6% of admissions during the study period.

PROGRESSION FROM COLONIZATION TO CDI

None of the 9 colonized patients went on to develop CDI during the study period. However, one of these patients was lost to follow-up, with their last hospital visit (inpatient or outpatient) recorded on September 13, 2017. Loss to follow-up in a cohort study may lead to bias and loss of statistical power, especially once the follow-up rate drops below 80%⁸². With an incomplete follow-up rate of 11.1% among the colonized patients in our study, it would have unlikely introduced any bias or impacted our ability to draw firm conclusions about predictors of progression for CDI.

RISK FACTORS FOR *C. DIFFICILE* COLONIZATION

Similar to the majority of studies in our scoping review that evaluated predictors for *C. difficile* colonization, we did not find an association between colonization status and age, gender or antibiotic exposure. When evaluating the entire cohort for the dichotomous outcome (i.e. colonized vs. never-colonized), univariable logistic regression did not identify any baseline characteristics that were predictors of colonization. The variables used in the limited multivariable analysis were therefore chosen based on risk factors for colonization that have been identified in the adult literature (previous CDI episode, use of corticosteroids)^{10,47,48} and

additional risk factors for CDI that been identified in the pediatric literature (enteral feeds) ^{31,32}, controlled for age, sex and the presence of other comorbidities. Hematologic malignancy was also chosen because the immunosuppressive nature of the chemotherapy regimens, resulting in more frequent episodes of febrile neutropenia necessitating hospitalization and antibiotics. The multivariable analysis was unable to identify any predictors significantly correlated with *C. difficile* colonization and the overall test for model significance (Omnibus Test of Model) was not statistically significant. The Omnibus test compares whether the explained variance in the new model (with explanatory variables included) is significantly greater than the baseline model. By using a χ^2 -test to determine if there is a significant difference between the Log-likelihoods of the baseline model and the new model, it can determine if the new model is an improvement over the baseline model (i.e. departure from the null hypothesis). All assumptions for use of the logistic regression were confirmed, including a linear relationship between the logit of the outcome and continuous predictor variables, lack of influential values in the continuous predictor variables and lack of multicollinearity between variables (none of the standard errors for beta-coefficients exceeded 2). It has been suggested that 10 outcome events per variable in a multivariable logistic regression are required to adequately power the analysis to detect an effect⁸³. In our study there were 9 colonized individuals and 65 who were never colonized. Therefore, the 9 cases would not provide an adequate number of events to accurately interpret the 4-variable multivariable logistic regression.

When data from the repeated measures (i.e. repeated admissions) were used for the 8 patients who had both positive and negative screening swabs, univariable logistic regression analysis using the GEE model was used to determine if various baseline characteristics or episode-dependent variables were correlated with *C. difficile* colonization. Malignancy type (hematologic

vs. solid tumor) was statistically correlated with developing *C. difficile* colonization. The two models utilize different patient populations, which may explain the differing results. In addition, the smaller sample size in this analysis is reflected in the larger confidence interval for the odds ratio that nearly overlaps 1 ($OR_{GEE}=3.64$, 95% CI (1.32,9.98) vs. $OR_{SLR}=0.75$, 95% CI (.17, 3.27). The variables used in the multivariable analysis were again chosen based on risk factors for colonization that have been identified in the adult literature (previous CDI episode, recent hospitalization and recent antibiotic use)^{10,47,48}, controlled for age and type of malignancy.

CONTRIBUTION TO THE LITERATURE

Our study adds to the current body of literature in a number of ways. First, it is the first study that provides longitudinal data across numerous admissions to hospital among pediatric oncology patients. Though Chiesa et al. collected longitudinal data over a 12-week period, they focused solely on children with acute lymphoblastic lymphoma who were on maintenance chemotherapy in a pediatric oncology clinic. Additionally, though a large proportion of admissions did not have *C. difficile* colonization data available, our study included a larger number of tested admissions than other studies identified in our scoping review. Not only does our study capture a broad group of oncology patients, the cohort size is larger than most studies from our scoping review, and the follow-up duration is longer.

This is the only study among pediatric oncology patients to detect *C. difficile* colonization using rectal swabs instead of stool samples. Though these swabs are easy to obtain and do not rely on the patient to have a bowel movement within the first 72 hours of admission, there may be a trade-off in the ability to detect *C. difficile*. The colonization rate in our study ($9/74 = 12.2\%$)

compared to the pooled colonization rate in the scoping review ($86/365 = 23.6\%$) was statistically different ($OR=0.45$, $95\% \text{ CI } (0.21, 0.94)$, $p=0.033$).

LIMITATIONS

Dataset

The 74 patients included in our cohort were admitted on 279 occasions for which we did not have *C. difficile* colonization results available. More than half of these missed opportunities ($n=161$, 57.7%) resulted from collected screening swabs not being tested because they were not transported to the research laboratory. Of the remaining 116 admissions, swabs were not obtained or were inappropriately obtained (i.e. after 72 hours of admission). On 45 occasions (38.8%), swabs were collected beyond 72 hours of admission. We had planned to exclude these from our analyses because any positive result may have reflected hospital-acquired colonization instead of colonization at the time of admission⁸. However, all 45 swabs were negative, suggesting the true colonization rate in our cohort may have been lower than what was observed. A sensitivity analysis including these swabs was not pursued when assessing for predictors of colonization. Had these admissions been included in the simple logistic regression analysis, the larger sample size would have generated narrower confidence intervals but it is unlikely to have altered the effect estimates.

Predictors of *C. difficile* Colonization

In addition to risk factors evaluated in the studies from our scoping review, we chose predictor variables based on adult literature looking at *C. difficile* colonization as well as the pediatric CDI literature. Though adult studies investigating risk factors for *C. difficile* colonization were not focused on the oncology population^{10,47-49,84}, based on our current understanding of the

development of colonization it would be reasonable to assume these risk factors would be shared across different patient populations. Risk factors from the pediatric CDI literature was sought out because there may be additional patient characteristics that are common or specific to the pediatric population such as the use of gastrostomy and jejunostomy tubes. There were risk factors from both sets of literature pertaining to underlying medical conditions such as chronic renal insufficiency, underlying bowel disease and gastrointestinal tract surgery that we did not include in our analysis because the number of patients affected was very limited.

Previous hospitalization has been quantified differently across studies^{10,19,47-49}. It has ranged from hospitalization within 30 days¹⁹ to hospitalization within the last 12 months⁴⁸. In addition to quantifying healthcare exposure as the number of hospitalizations, other studies have also used the number of outpatient visits³⁶, and the number of days of hospital admission⁵¹. We chose to count the number of days hospitalized over two time periods, 3 months and 6 months. Though this has the ability to more accurately reflect the time spent in hospital, it may not appropriately capture the cumulative aspect of hospitalizations if recent hospitalizations varied significantly in duration (i.e. were very brief or prolonged).

Similarly, recent antibiotic exposure has also been quantified using a variety of measures^{50-52,84,85}. Antibiotic use was reported by certain classes of antibiotics, or any type of antibiotic over variable durations of time up to 3 months. Therefore, we created a composite antibiotic risk score, defined as days of therapy (DOT) of certain antibiotics that have been associated with either *C. difficile* colonization or CDI in the literature (i.e. third generation cephalosporins, beta-lactam/beta-lactamase inhibitor combinations, carbapenems, fluoroquinolones and clindamycin). Since DOT data was obtained from the hospital's electronic medical record, the ability of this

composite score to comprehensively capture the cumulative exposure to antibiotics was limited as we could only accurately capture inpatient antibiotic use (which was also dependent on variation in hospitalization duration) but not outpatient antibiotic use.

Statistical Limitations

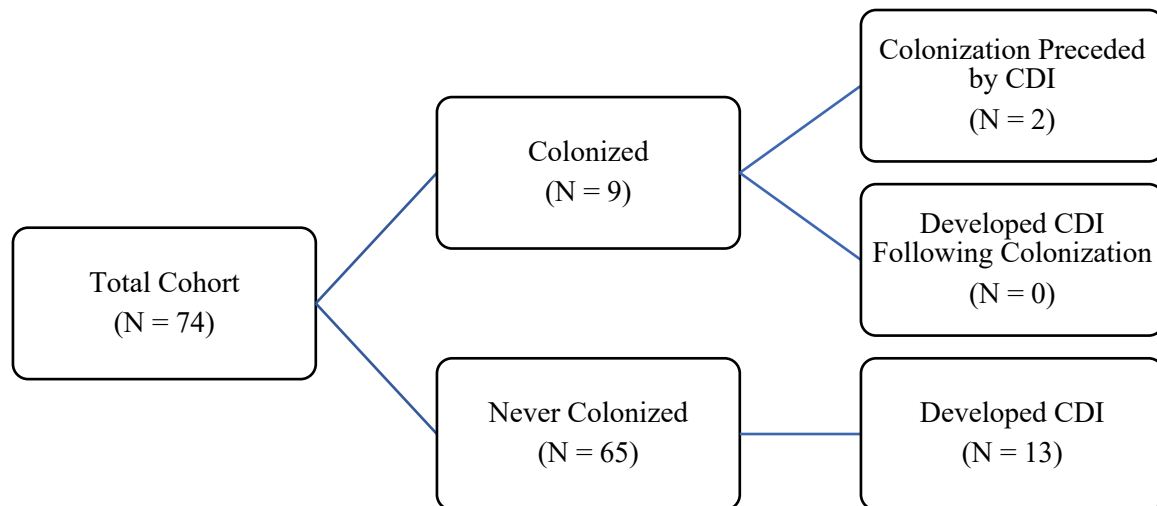
Longitudinal data analysis allows us to study changes in *C. difficile* colonization status and predictor variables over time. However, in order to appropriately analyze the data obtained by repeated measurements on the same patient at each hospital admission, a model that can account for the correlation between measurements is required. A simple logistic regression analysis of a binary outcome would generate incorrectly underestimate standard errors of the estimated parameters. The Generalized linear models (GLM) was developed to overcome this challenge, including the extension to the Generalized Estimating Equations (GEE) analysis method⁸⁶. GEE can accommodate a variety of models including linear regression, logistic regression, Poisson regression and proportional odds. It also offers advantages over mixed models by fitting population-average models and allowing for weaker distributional assumptions⁸⁶.

The outcome of interest in our study was binary (colonized vs. not colonized). Therefore, the GEE model specified included a logit link function and binomial distribution for logistic regression. The working correlation matrix can be independent, exchangeable, first-order autoregressive and unstructured. An independent correlation model was chosen *a priori* as the most commonly used model⁸⁷. In a larger cohort study, with over 100 clusters, the choice of correlation matrix is unlikely to affect the regression coefficient estimates⁸⁷. However, given our smaller cohort we repeated our analysis using other working correlation matrices, and the independent correlation model yielded the best fit. The quasi-likelihood under the independence

model criterion (QIC) is a modification of Akaike's information criterion (AIC) and is commonly used as an indicator of model fit and proposed as a criteria to aid in selecting a working correlation matrix^{88,89}.

Our GEE analysis was limited to 8 colonized patients who had positive and negative swabs over the course of the study. When the clustering units (in our case, the individual patients) is below 30 or 40 for binary outcomes, the GEE can produce a downward- biased standard error estimate⁹⁰. We tested three models, and none revealed any association between recent hospitalization and/or antibiotic use with *C. difficile* colonization after controlling for age, hematologic malignancy and previous CDI. Therefore, had we included all 74 patients (with the vast majority never developing colonization), the 95% CIs for the estimated aORs would have been narrower and less biased, however the *p*-values for the estimated aORs would have likely remained not statistically significant.

FIGURE 1 – Distribution of *C. difficile* colonization and CDI



PART V: CONCLUSIONS & FUTURE STEPS

Limited literature suggests *C. difficile* colonization rates can range from 10% to 40% among the pediatric oncology population. During our 18-month study of a diverse pediatric oncology cohort receiving care at a tertiary-care pediatric hospital, 12.2% of patients were asymptotically colonized on at least one admission occasion. It is uncertain how our colonization rate compares to other studies since prevalence was determined differently across studies. However, there are a number of environmental and microbiological testing variables that could explain a true lower colonization rate among our cohort. Similar to the majority of studies in our scoping review, there was no statistical association between *C. difficile* colonization status and age, gender or antibiotic exposure. Our cohort was small compared to other cohorts described in the adult literature on *C. difficile* colonization, which limited the number of variables explored in both of the simple logistic regression and GEE multivariable analyses. It remains unclear which patient and epidemiologic characteristics are predictors for colonization. Furthermore, we were unable to determine risk factors for progression from colonization to CDI as none of our colonized patients subsequently developed CDI.

Future studies assessing *C. difficile* colonization in this population will require a variety of adjustments to enhance the reliability of calculated admission prevalence rates and to allow for comparison between studies. In addition to conducting studies over similar durations, patients would need to be tested during the same time interval after admission (i.e. within 72 hours of admission in order to exclude those who acquired colonization during their admission). Given that not all patients are able to provide a stool sample, screening using rectal swabs would hopefully capture more patients. Added quality-control measures to ensure swabs are obtained at

all opportunities (i.e. consistently obtained within 72 hours of admission) and adequate fecal staining has been achieved on each swab will also be needed. Many oncology patients are receiving antibiotics at the time of admission, therefore enteric bacterial loads or biodiversity may not be more advantageous than visual inspection for fecal staining. Though a reference standard for microbiological detection of *C. difficile* colonization does not currently exist, utilizing a molecular method such as PCR offers a sensitive option that has been validated for testing of rectal swabs.

With regards to study methodology, utilizing a prospective cohort approach with continuation of screening on a fixed schedule (including both the inpatient and outpatient settings) may better delineate the duration of colonization as well as where colonization may occur. Including multiple study sites would also assist in capturing a larger cohort. A study to measure other epidemiological features such as time-to-colonization from the time of cancer diagnosis would also provide additional valuable information regarding the natural history of *C. difficile* colonization in this patient population.

At this time, it remains to be determined which patients in the pediatric oncology population are at highest risk for *C. difficile* colonization, and which colonized patients may be more likely to progress to CDI.

APPENDIX

APPENDIX 1 – Data Collection Form

Patient ID

Date of swab sample testing

Positive CD Result (Yes/No)

	Information To Be Collected	Format	Source
Demographic Information	Age at time of admission	Years (2 decimal points)	Medical Chart
	Gender	M/F	
	Other underlying conditions	Text	
	Date of admission	DD/MM/YYYY	
	Date of discharge	DD/MM/YYYY	
	Reason for admission	Chemotherapy, Infection, Other	
	Previous hospital admissions (including other hospitals)	Yes/No If Yes: Date of Admission (DD/MM/YYYY) & duration (Days)	
	Previous ICU admissions	Yes/No	
Cancer Treatment Information	Malignancy	Hematologic/Solid Text: name of malignancy	Medical Chart
	Chemotherapy protocol	Text	
	Previous high-dose corticosteroids	Yes/No	
	Use of enteral feeds	Yes/No	
Current Antibiotic Use	Antibiotics being used as treatment	For Each Antibiotic Class: Yes/No If Yes: Text: name of agent Start & Stop Dates: DD/MM/YYYY	Medical Chart
Previous Antibiotic Use	Previous use of 3 rd generation cephalosporins, beta-lactam/beta-lactamase inhibitor combinations, carbapenems, fluoroquinolones,	Yes/No If Yes: Days of Therapy in last 3 months	Medical Chart

	clindamycin in the last 3 months		
Stomach Acid Suppression	Proton Pump Inhibitors, H2-Receptor Antagonists	No/H2RA/PPI	Medical Chart
Diarrheal Episodes (Current Admission)	Date of onset	MM/DD/YYYY	Medical Chart
	Amount of diarrhea	Number loose bowel movements/day	
	Duration	Number of days	
	Other Symptoms	Fever (Yes/No) Abdominal Pain (Yes/No) Ileus (Yes/No)	
	Other Signs	Pseudomembranes (Yes/No) Pseudomembranous Colitis (Yes/No) Toxic Megacolon (Yes/No)	
	Other reasons for diarrhea	Laxatives (Yes/No) Recent travel (Yes/No) Other diagnosed infection (Yes/No, Name of pathogen)	
Previous <i>C. difficile</i> Infection	Documented on IPAC Surveillance Data	Yes/No (If Yes, Date DD/MM/YYYY)	IPAC Surveillance Data
	From Microbiological Results	Yes/No (If Yes, Date DD/MM/YYYY)	Medical Chart
ARO Screening	Other positive ARO results	Yes/No If Yes: Date: DD/MM/YYYY Text: Name of ARO	Medical Chart

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