PREVALENCE AND TRANSMISSION OF ASYMPTOMATIC *C. DIFFICILE*

EVALUATION OF THE PREVALENCE AND TRANSMISSION OF ASYMPTOMATIC *CLOSTRIDIOIDES DIFFICILE* CARRIAGE IN THE HAMILTON IN-PATIENT SETTING USING MULTI-LEVEL MODELLING

By SYDNEY GEORGE, B.Sc.

A Thesis Submitted to the School of Graduate Studies In Partial Fulfilment of the Requirements for the Degree Master of Science In Health Research Methodology

MASTER OF SCIENCE (2020) **McMaster University**

Health Research Methodology **Hamilton, Ontario, Canada**

TITLE: Evaluation of the Prevalence and Transmission of Asymptomatic *Clostridioides difficile* Carriage in the Hamilton In-Patient Setting Using Multi-Level Modelling

AUTHOR: Sydney George, B.Sc.

SUPERVISOR: Dr. Dominik Mertz, MD, MSc, FMH

NUMBER OF PAGES: xi,71

LAY ABSTRACT

C. difficile infection (CDI) is a severe infectious disease. Patients with asymptomatic *C. difficile* present a risk to others as they can contribute to the spread and development of hospital-associated CDI. We are currently unsure of the proportion of adult in-patients colonized with asymptomatic *C. difficile*. Identifying these carriers early on in their hospital stay is imperative to reduce CDI rates in health care settings. Our study objectives were to determine the best screening strategies to identify asymptomatic carriers, identify risk factors for carriage, and understand the transition from asymptomatic *C. difficile* to symptomatic CDI. We demonstrated that being female, being recently hospitalized or previously having CDI may increase a patient's risk of being an asymptomatic carrier. Also, timely screening throughout a hospital stay in addition to admission screening helped identify more colonized in-patients. Lastly, we determined that 1 in 5 carriers would go on to develop symptomatic CDI infection.

ABSTRACT

Background: *C. difficile* is one of the primary infectious causes of morbidity and mortality in Canada. Colonized patients can pose a risk to others as a factor in the transmission and development of hospital-associated *C. difficile* infections. Despite immense efforts and resources invested in the reduction in *C. difficile* transmission within Canada and Hamilton Health Sciences – further reduction in these rates are unlikely, and novel screening strategies are imperative in this field of study.

Methods: This project was a retrospective cohort study of adult in-patients admitted to either The Juravinski, Hamilton General, or St. Joseph's Healthcare Hamilton Hospitals from January to April 2018 and September 2018 to August 2019. MSRA/VRE swabs were collected during admission or through universal point prevalence screening and subsequently tested for colonization.

Results: From the 1056 patients in the data sample, 72 were colonized with asymptomatic *C. difficile* resulting in a prevalence rate of 6.81%. In-patient point prevalence screening strategies identified more carriers than admission swabs alone ($p < 0.001$). Risk factors for colonization on admission were being female (OR 2.66, 95% CI 1.02-8.33) and previous CDI (OR 4.76, 95% CI 1.49 – 13.86). During hospitalization, risk factors for colonization were previous CDI (OR 4.75 95% CI 2.14-9.94) and recent hospitalization within the last 12 months (OR 2.35, 95% CI 1.30-4.42). The multi-level Cox PH model identified those with a recent hospitalization (OR 2.21, 95% CI 1.32 – 3.73) and those with previous CDI (OR 2.40, 1.34 – 4.30) were twice as likely to develop asymptomatic C. difficile colonization throughout hospitalization.

Conclusion: The addition of universal point prevalence screening in addition to admission screening helped identify more than double the amount of carriers in the population. Moreover, a previous hospitalization, previous CDI, and being female may indicate patients at the highest risk of colonization.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Dominik Mertz, for his constant support, guidance, and mentorship throughout my Health Research Methodology studies. Secondly, I would like to thank my committee members Drs. Marek Smieja, Robby Niewulaat, and Allison McGeer for their feedback and direction throughout the completion of my thesis project. I would also like to thank Julia Maciejewski for processing the laboratory data needed for my thesis. Lastly, I would like to thank my family and friends for their constant and unwavering support over the years towards my academic pursuits.

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LIST OF ALL ABBREVIATIONS AND SYMBOLS

CA-CDI	Community Associated *Clostridioides difficile* Infection
CCCNA	Cell Culture Cytotoxicity Neutralization Assay
CDC	Centers for Disease Control and Prevention
CDI	*Clostridioides difficile* Infection
EIA	Enzyme Immunoassay
GEE	Generalized Estimating Equation
HA-CDI	Hospital Associated *Clostridioides difficile* Infection
HHS	Hamilton Health Sciences
HRLMP	Hamilton Regional Laboratory Medicine Program
LAMP	Loop-Mediated Isothermal Amplification
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NAP1	Nucleosome Assembly Protein-1
PCR	Polymerase Chain Reaction
VRE	Vancomycin-Resistant *Enterococci*

DECLARATION OF ACADEMIC ACHIEVEMENT

I, Sydney George, declare my thesis to be my own research work. I am the sole author of this thesis document and was involved in all stages of the research project under the supervision of Dr. Dominik Mertz. The following individuals contributed to the editing and refinement of my thesis work and acting as the members of my thesis committee: Dr. Dominik Mertz, Dr. Marek Smieja, Dr. Robby Nieuwlaat and Dr. Allison McGeer. Julia Maciejewski processed laboratory results for data collection. To my knowledge, the content of this document does not infringe on any copyrights.

PART I: INTRODUCTION & NARRATIVE REVIEW

An Introduction to *Clostridioides Difficile*

Clostridioides difficile (*C. difficile*) is a gram-positive bacterium and a spore-forming obligate anaerobe first described as *Bacillus difficilis* by Hall and O'Toole in 1935(1). Up until the early 2000s, infection by *C. difficile* was not viewed as a threat to public health and a treatable complication of antimicrobial therapy(2). However, *C. difficile* infection (CDI) has re-emerged as a severe infectious disease with increased complications(2). Currently, CDI is the leading cause of antibiotic-associated diarrhea in Europe and North America and affects more than 300,000 hospitalized patients yearly in the United States(2). Moreover, there were 20, 623 cases of health care associated CDI from 2009-2015 in Canada(3). *C. difficile* became a notifiable disease under national surveillance in 2009 through the Canadian Notifiable Disease Surveillance System (CNDSS)(4).

C. difficile is commonly found in water, air, and soil – as well as human feces and hospital surfaces(5). Spores of *C. difficile* are dormant cells and are resistant to high temperatures, ultraviolet light, harsh chemicals, disinfectants, and antibiotics(6). *C. difficile* bacterium is known to colonize the colon primarily due to the large bowel being an anaerobic environment, and antibiotic medication can eliminate the absence of competitor flora(7). Following or during colonization, vegetative *C. difficile* bacterium releases toxin A (TcdA) and toxin B (TcdB)(8).

TcdA acts primarily on the intestinal epithelium, causing fluid secretion, general inflammation, and necrosis of tissue, whereas TcdB acts as a potent cytotoxin(8). The clinical manifestations of *C. difficile* infection can range from mild diarrhea to life-threatening toxic megacolon or pseudomembranous colitis(9). The severity of CDI depends on the virulence (e.g. NAP1) of the pathogen and host characteristics(10). *C. difficile* is a common nosocomial pathogen, with reservoirs regularly being patients and contaminated environments(7).

In disease-endemic settings, the prevalence of colonized but asymptomatic hosts is typically higher than symptomatic CDI cases(11). Therefore, asymptomatic *C. difficile* colonized patients may be an essential infection reservoir and a source of transmission(11). Prevalence of *C. difficile* colonization differs between populations, depending on several patient, environmental, and pathogen characteristics(11). Nonetheless, it is imperative to understand vehicles of transmission for *C. difficile* in healthcare settings – which remains an ongoing challenge to the world of infection prevention and control.

Transmission of CDI

C. difficile is transmitted via the oral-fecal route(5). Spores are the primary infectious vehicle of *C. difficile*, as vegetative *C. difficile* bacterium is unlikely to

survive in an oxygenated environment and outside of the acidic environment of the stomach or the host in general(12).

CDI is differentiated between hospital-acquired and community acquired, where;

a) Hospital-Acquired CDI (HA-CDI): The symptoms of CDI were not present on or within 72 hours of admission, or CDI symptoms are present at the time of admission however are related to a previous admission to a healthcare facility within the last four weeks (13).

b) Community-Acquired CDI (CA-CDI): The symptoms of CDI were present on admission or developed within < 72 hours after admission, with no previous hospital admission within the last four weeks (13).

C. difficile has primarily been considered a hospital-acquired infection, and the Centers for Disease Control and Prevention (CDC) has estimated that 94% of all CDI is related to healthcare setting exposure(14). A population-based study, however, found that a substantial proportion of CDI was community-acquired, and other surveillance studies have found the same percentage of community-acquired CDI cases among all CDI cases(15-17). Moreover, community-acquired CDI affects previously considered low-risk populations such as younger patients with no antibiotic exposure and recent hospitalization(18). A study by Kuntz et al

identified that 17% of CA-CDI patients did not have any of the traditional risk factors for CDI (19).

Furthermore, due to *C. difficile* spores' antibiotic resistance, they can remain in the gastrointestinal tract, making recurrent disease following treatment likely(20). Recurrence of symptoms after effective treatment of CDI is widespread, and historically recurrent CDI occurs in 20-30% of patients(20). This rate of recurrence may be higher since the appearance of the virulent NAP1 epidemic strain(21).

Epidemiology & Disease Burden of *C. difficile* in the Canadian Context

C. difficile is one of the primary infectious causes of morbidity and mortality in Canada and is the most frequent cause of infectious diarrhea in hospitals and long-term care facilities(22). Between 2005-2015 alone, there were approximately 40,000 new cases of *C. difficile* infection in Ontario – half of which were acute care hospital-associated(23). Since 2002, there has been an increase in CDI incidence and severity in North America(24). A Quebec study reported that between 1991 and 2003, there was a 330% increase in CDI cases per 100,000 people(23). In 2011, the incidence rate of CDI among Canadian provinces was 535 per 100,000 admissions (23).

Moreover, 30-day mortality and the proportion of severe complications (development of megacolon, colectomy, for example) increased between 1991 and 2003(2). *C. difficile* disproportionately affects the elderly, with 93% of CDI deaths occurring in persons older than 65 years(2). The disproportionate prevalence of cases in the elderly population is of concern with a current rising average age of Canadians.

The incidence of community-acquired CDI cases has increased as well. Khanna et. al demonstrated that community-acquired CDI raised 5.3 fold in 10 years in Olmsted County, Minnesota(25). Community-acquired CDI also tended to affect a different population in comparison to hospital-acquired CDI, with most cases being: younger (median age 50), likely to be female and with fewer comorbidities than patients with hospital-acquired cases(2). A study by Reveles et. al found an increase of community associated CDI from 8.3% to 26.7% of patients between 2002 to 2014(26). Patient characteristics of community-acquired cases were: less likely to have a severe infection, less likely to be on antibiotic treatments, and less likely to have cancer than hospital-acquired CDI patients(2).

Researchers have begun to explore why there is an emergence of virulent CDI, and it is thought to be attributed to hypervirulent strains(27). It has been reported in the literature that the ribotype NAP1/B1/027 strain of CDI may be

more virulent compared to other pathogenic isolates, and ribotype 078, 126, 056, and 018 may contribute to more severe CDI outcomes as well(2).

CDI has also been associated with substantial costs to the Canadian health care system. A study in Ontario has shown that acquiring a *C. difficile* infection increases the median length of an in-patient stay by six days(28). The mean attributable costs of a CDI infection per hospitalized patient ranged from CAD $ 10,961 to CAD $ 36,960(29). Another study estimated that mean attributable 1-year costs were $13,217 (95%CI, $12,062-$14,388) unadjusted for survival and $10,700 (95%CI, $9,811-$11,645) adjusted for survival(29). A Canadian model estimated a combined 37,900 cases of CDI in the hospital and the community in 2012, had a societal cost of $ 281 million, of which 92% were in-hospital costs(30). The re-emergence of CDI affects a vulnerable sector of the population and has resulted in more frequent surgical complications, severe outcomes of disease, increased health care costs, increased mortality rates, and will be a significant contributor to economic burden in the years to come without interventions.

Asymptomatic *C. difficile* Colonization

Asymptomatic *C. difficile* colonization is defined as a condition where a patient has detectable concentrations of *C. difficile* or its toxin gene in the

absence of symptoms of CDI (diarrhea, and colonoscopic or histopathologic findings consistent with pseudomembranous colitis)(11). Asymptomatic *C. difficile* colonized patients may be important reservoirs of infection. Colonized patients can present a risk to others as a factor in the transmission and development of hospital-associated *C. difficile* infections, especially with emergent hypervirulent strains being of concern. 29% of isolates from hospital associated CDI were related to isolates obtained from colonized patients (31). More than 30 years ago, 79% of hospital-associated CDI were reported to be linked to asymptomatic carriers(32). Literature also suggests that asymptomatic *C. difficile* colonization with non-toxigenic strains may be protective of disease progression to symptomatic CDI due to a coordinated immune response to *Clostridioides* toxins(11, 33). Nonetheless, it is essential to establish the contribution of asymptomatic patients as vehicles of disease transmissions

After exposure to *C. difficile*, an estimated two-thirds of patients who acquire the organism will become asymptomatic carriers(34). The terms asymptomatic carriage or asymptomatic colonization are used interchangeably within the literature, with the term carriage used more often to refer to persistent colonization(11).

Epidemiology of Asymptomatic *C. difficile* Colonization

Asymptomatic *C. difficile* colonization is more common than symptomatic CDI(11). However, the prevalence of colonization depends significantly on the patient population and environment – with considerably different prevalence rates between groups. Due to the nature of asymptomatic carriage, it is difficult to understand its clinical implications fully and testing patient populations for colonization is required. The highest colonization rates are in infants during their first year of life – who are commonly colonized and have a range of carrier rates being between 18-90%(11). Pediatric oncology in-patients have been found to have prevalence rates of colonization approximating 25%(35). Moreover, cystic fibrosis patients have colonization rates between 18-47%(11). Healthy adults in the general population have been found to have prevalence rates from 0-15%(11). A large Canadian study found that in a mixed population of hospital admissions, colonization rates were at 4.05%, and other literature has suggested the in-patient population prevalence rate to be between 4-29%(11, 36). The significant heterogeneity in presented prevalence rates is probably due to differing populations, screening strategies, environment and clinical settings, and detection methods.

Risk Factors for *C. difficile* Colonization

Risk factors for *C. difficile* colonization in adult in-patient populations have been identified in recently published literature. Risk factors for *C. difficile* colonization include a previous hospitalization within the last 12 months, medication therapies including proton pump inhibitors, H2 blockers, chemotherapy, cephalosporin use during admission and presence of toxin B antibodies – which have also been suggested to be protective against the development of CDI(11, 36).

Prevalence within the healthy adult population remains considerably low. However, the prevalence increases significantly once a patient is within the healthcare system. For example, a study found that long-term care homes have a rate of colonization between 0-51%, and a high prevalence of asymptomatic *C. difficile* carriage is common among healthcare workers, patients in rehabilitation centres or hospitals in-patients(11). A study by Kong et al. reported that a previous history of CDI and the use of corticosteroids are risk factors of *C. difficile* colonization(36). Lastly, patients with multiple comorbidities have been associated with higher *C. difficile* colonization as well(37). Understanding the landscape of potential risk factors may help guide screening strategies for *C. difficile* colonization detection.

Laboratory Detection of *C. difficile*

The process of laboratory detection for *C. difficile* involves detecting toxins A and B of toxigenic *C. difficile* in the stool(38). There are a variety of diagnostic methods available, each with their own respective advantages and disadvantages. Currently, the gold standard for detecting *C. difficile* toxin is cell culture cytotoxicity neutralization assay (CCCNA) and anaerobic toxigenic culture (TC)(38). Enzyme immunoassay (EIA) is another widely used detection method. The advantages of the tests are that CCCNA has high sensitivity, and EIA has a rapid turnaround, has an associated low-cost and is a simple technique(38). However, CCCNA is time-consuming, TC is slow with a 48-72 hour turnaround, and EIA has low sensitivity with ranges of 50-90%(39). PCR is another commonly used tool for detecting *C. difficile* toxins, with rapid turn around and high sensitivity, however, with PCR needing more specific equipment – the use of PCR as a primary detection method for *C. difficile* would be impractical for many clinical laboratories(38). Loop-mediated isothermal amplification (LAMP) is recently another diagnostic tool used for *C. difficile* detection, which has the benefit of being both sensitive (98%) and specific (98%) with a rapid one-hour turnaround time and is comparable to both PCR and EIA methods(40). Literature, however, debates on the importance of which toxin LAMP targets. LAMP targeting toxin A may not be ideal as toxin B is proven to be more virulent and there is the existence of toxin A-negative *C. difficile* strains, which studies have shown to make up 21.4% of CDI strains and may also present a challenge (41).

Nonetheless, LAMP may be a cost-effective, rapid and reliable diagnostic method to use for *C. difficile* toxin detection.

Testing Strategies for *C. difficile* Colonization

Although the role of asymptomatic *C. difficile* carriers as vehicles for transmission is poorly understood, literature using whole genome-wide sequencing and molecular typing suggests they may play an essential role in infection spread. Preliminary research has suggested that early identification and intervention of asymptomatic colonized patients with *C. difficile* may reduce transmission. Longtin et. al 2016 demonstrated that universal admission screening for *C. difficile* carriage and isolation in conjunction with contact precautions of identified carriers reduced hospital CDI by 62%, and estimated that 60 cases of acute CDI were prevented(42). In consideration, however, mass screening techniques of all newly admitted patients for *C. difficile* can be time-consuming and costly, while putting an additional workload strain on hospital microbiology labs.

Patients high-risk for MRSA/VRE carriage have previous hospitalization as a risk factor, and systematic screening of this population is recommended. Similarly, previous hospitalization within three months is a risk factor for *C.*

difficile carriage(11). Therefore, one could conclude that patients who are high risk for MRSA/VRE carriage may be high-risk for *C. difficile* colonization, and repurposing MRSA/VRE swabs may be a method of screening patients that is well integrated into existing processes. A study with 31 participants determined that a screening strategy based on high-risk MRSA/VRE carriers could identify 74% of all *C. difficile* carriers(43). Another study revealed that 9.5% of inpatients were carriers upon admission; however, routine weekly screening identified another 7.2% of colonized patients – who acquired *C. difficile* colonization after hospital admission(33). Another study had reported an increase of 50% in colonization rates throughout a hospital stay, despite a relatively low colonization rate amongst admitted patients (2.1%)(32). Therefore it is imperative that both admission and point prevalence screenings are taken into account for developing a screening method for asymptomatic *C. difficile* carriage detection in order to capture all colonized patients.

Asymptomatic *C. difficile* colonization progression to Symptomatic CDI

While the disease process of CDI is well understood, the role of asymptomatic *C. difficile* colonization and its development into symptomatic CDI has a weaker understanding. The risk of asymptomatic carriage and its progression to symptomatic CDI is highly variable, with heterogeneity relating to patient characteristics, bacterial factors and other extrinsic risk factors(44).

Patients who had acquired asymptomatic *C. difficile* colonization were less frequently colonized with the hypervirulent NAP1 strain, in comparison to those with symptomatic CDI infection – and may suggest strain may influence whether a patient is asymptomatic or symptomatic (11). Moreover, in a published systematic review, 21.8% of adult patients who were colonized upon admission developed CDI during their hospital stay – resulting in patients colonized having a higher risk of symptom development than non-colonized patients(45). Another study reported a rate of 2.1% of CDI development from colonization in a low-risk mixed patient population. Rates of CDI development from colonization may be much higher in populations such as hematopoietic stem cell transplant patients; a study found that 85.5% developed CDI in comparison to 17.2% of non-carriers (odds ratio (OR) 68.5, 95% CI 11.4-416.2) (46). Asymptomatic colonization has also potentially been considered a protective factor against CDI. A meta-analysis of 4 studies demonstrated overall that patients colonized with *C. difficile* had a lower risk of subsequently developing CDI(47). Furthermore, the evidence shows that patients colonized specifically by only non-toxigenic strains are protected from progressing to CDI, while those colonized with toxigenic C. difficile at admission progress to CDI more frequently (RR 5.86, 95% CI 4.21-8.16)(44, 47). Our goal is to assess the natural history of *C. difficile* carriage in in-patient populations in order to inform both future interventional studies and screening programs.

PART II: RETROSPECTIVE COHORT STUDY

Materials and Methods

INTRODUCTION

The estimation of the prevalence of asymptomatic *C. difficile* colonization in the adult in-patient population is relatively uncertain. Moreover, in order to reduce the rates of transmission of *C. difficile* within hospital settings, the identification of carriers early on in the in-patient process is imperative. Therefore a longitudinal retrospective cohort study was established to: 1) identify optimal screening strategies for asymptomatic carriers, 2) understand the risk factors for asymptomatic *C. difficile* carriage at admission and during a hospital stay, and 3) understand the transition from asymptomatic *C. difficile* carriage to symptomatic CDI as well as the natural history of the disease.

OBJECTIVES

Subproject 1: Screening, Admission & Point Prevalence

Primary Objective

To determine whether admission screening swabs of high-risk individuals for MRSA/VRE (done as part of routine MRSA/VRE surveillance) can identify the majority (>80%) of in-patients who were colonized with *C. difficile* before hospital entry, using routine universal point prevalence testing of the entire unit as the reference standard.

Secondary Objective

Estimate the incidence of *C. difficile* colonization acquisition during hospital stays on high-risk wards by documenting the proportion of individuals negative at admission who are later found to be positive for asymptomatic colonization.

Sub-Project 2: Symptomatic CDI & Transmission

Primary Objective

To determine the incidence of symptomatic *C. difficile* infection (CDI) amongst colonized patients.

INVESTIGATION PLAN

Summary of Study Design

This project was a retrospective cohort study of adult in-patients admitted to one of the study wards over eight months. High-risk in-patients routinely screened for MRSA/VRE upon admission or throughout routine, point-prevalence screening had their samples tested for *C. difficile* colonization. Amongst patients tested for *C. difficile*, a retrospective chart review was conducted to identify colonized in-patients and potential risk factors of interest for asymptomatic colonization and the development of symptomatic *C. difficile* infection.

Retrospective Cohort Study

This project was a retrospective cohort study of adult in-patients admitted to either The Juravinski, Hamilton General, or St. Joseph's Healthcare Hamilton Hospitals from January to April 2018, and September 2018 to August 2019. High-risk in-patients routinely screened for MRSA/VRE upon admission or throughout routine, point-prevalence screening had their swab samples tested for *C. difficile*. Among patients tested for *C. difficile*, a retrospective chart review was conducted to identify colonized in-patients and potential risk factors of interest for asymptomatic colonization and the development of symptomatic *C. difficile* infection.

Rationale for Study Design

In an observational cohort study, participants are selected based on exposure status and do not have the outcome of interest at baseline(48). Participants are grouped into either exposed or not exposed at study initiation and are followed-up over a specified time for assessment of the occurrence of the outcome of interest(48). In a retrospective cohort study, outcomes of interest have occurred in the past, and data is collected from databases or health care records. A cohort study is an advantageous design for assessing causal relationships in comparison to other observational study designs, such as case-control studies, due to the temporal sequence of risk factors recorded before the occurrence of the outcome.

Moreover, a cohort study allows researchers to examine multiple outcomes for a given exposure and determine the incidence and prevalence rates of disease. In particular, retrospective study designs are more cost-efficient and shorter than prospective study designs. Limitations of a retrospective cohort study typically include limited control the investigator has over data collection, resulting in sometimes inaccurate or incomplete study data. A cohort study design allows for the evaluation of the natural history of colonization and calculation of incidence and prevalence of both asymptomatic colonization and symptomatic *C. difficile* infection.

Also, given the exploratory nature of the project, a cohort study allows for examining several risk factors of interest (e.g., antibiotic exposure, duration of hospital stay, comorbidities of interest) for asymptomatic *C. difficile* colonization. Lastly, repurposing routine MRSA and VRE swabs for *C. difficile* testing allowed for a reasonable period for data collection for a Master's thesis. Therefore, a retrospective cohort study was the ideal study design for this project.

Prospective Cohort Study

Prospective cohort studies involve subject recruitment and exposure data collection before participants developing any outcomes of interest (at baseline) with comparison to non-exposed participants(48). Over time, participants are followed-up through mediums such as mail questionnaires, phone or in-person interviews, physical examinations, or laboratory tests. Disadvantages of a prospective cohort study are that they require long follow-up periods while waiting for outcomes to develop, are vulnerable to high study attrition rates, and can be quite costly. Accompanying the advantages of a prospective cohort study includes increased control an investigator has over data collection. Furthermore, a prospective study design does not deal with recollection bias – which is a common disadvantage of retrospective cohort studies.

In this project's context, however, prospective follow-ups of a patient could become lengthy, especially if they have extended hospital stays and costly as

well – which would not have been suitable for a timeline and budget of a Master's thesis project. A benefit of a prospective study would have been that laboratory samples only needed to be stored for select patients which could have provided relief in terms of storage capacity on the lab. However, adequate data collection in the timeline of a Master's thesis would not have been feasible.

Case-Control Study

Case-control studies involve identifying patients with the outcome of interest at baseline and then reviewing patient history retrospectively to identify potential risk factors(48). A case-control study design consists of a comparison between patients with the outcome of interest and a control. A case-control study is a robust observational study design to determine the importance of a predictor variable in the presence or absence of an outcome of interest.

For the project to be framed as a case-control study, cases and controls would have needed to be matched on variables believed to be confounders, in order to reduce confounding in the sampling design and to increase study efficiency by ensuring cases and controls have similar distributions of confounding variables(49). However, due to limited research on asymptomatic colonization, it is hard to have adequate information on what may be effective confounders of interest to match patients on. Therefore, a retrospective cohort study was the ideal study design.

Study Duration and Enrollment

Participants were considered for study inclusion if they were admitted to general medicine at Hamilton General and The Juravinski Hospitals, or hematology-oncology at The Juravinski Hospital or general medicine and nephrology wards at St Joseph's Healthcare Hamilton between April 2018 and August 2019 (Appendix A). Follow-up information was collected from the date of admission to date of discharge. Based on estimates from the DECEnCY Study Protocol, it was expected that 1000 swabs and accompanying clinical data would be collected from during this time frame with an expected prevalence of colonization of 5-10%.

Study Population

Inclusion Criterion

A patient was included if they fulfilled all of the following criteria: admitted as an in-patient, age > 18 years, MRSA/VRE screening swab collected at either hospital admission or during routine point prevalence screening.

Exclusion Criteria

The pediatric population < 18 years of age were excluded due to differing colonization rates.

STUDY PROCEDURES

MRSA/VRE Screening; Admission & Point Prevalence

The screening protocol for this project is centered around existing Infection Prevention and Control screening policies and procedures for MRSA/VRE at Hamilton Health Sciences and St. Joseph's Hospital Hamilton. Routine universal point prevalence screening occurs monthly on high-risk wards except during outbreaks, where screening occurs weekly. Admission screening for MRSA/VRE occurs for patients considered high-risk and those admitted to the ICU. High-risk MRSA/VRE patients were defined based on prior hospitalization within the previous 12 months and known history of MRSA/VRE colonization. Rectal MRSA/VRE swabs were collected within 72 hours of admission.

Moreover, previous hospital admission has also been found to be a significant risk factor for *C. difficile*, MRSA, and VRE colonization(50). Literature has suggested that a risk-factor screening strategy based on MRSA/VRE risk criteria would identify 74% of all *C. difficile* carriers(43). Therefore, it is plausible that individuals likely to be asymptomatic *C. difficile* carriers are similar to those routinely screened for MRSA/VRE, this assumption forms the basis of the screening strategy for this project.

Swabs for *C. difficile* Testing

Upon admission or throughout point prevalence screening, nurses collected MRSA/VRE rectal swabs using Copan eSwabs and submited the swabs in Amies transport medium to the microbiology laboratory as per usual practice. Point prevalence swabs for MRSA/VRE are conducted routinely once per month, and during outbreaks on a weekly basis. Next, swabs of interest based on hospital and ward were sent to the Infectious Diseases Research Laboratory at St. Joseph's Research Institute and screened by LAMP. Positive specimens were verified by PCR, and culture to detect colonization. The LAMP assay targets the *tcd*C gene which is a regulatory element in the gene cassette for *C. difficile* toxin production. PCR for toxin A or binary toxin was used to verify LAMP results. Rectal swabs, as used in the study context for MRSA/VRE surveillance, have close to 100% sensitivity and specificity in *C. difficile* detection, outperforming molecular testing from stool samples, and toxigenic culture of colonized patients(51).

Outcomes: Case Definitions for *C. difficile* Colonization & *C. difficile* Infection

C. difficile Colonization

A case of asymptomatic *C. difficile* colonization was determined by a positive LAMP assay for *tcdC* gene from rectal swab, and confirmed with a positive PCR result for toxin A/binary toxin. A case of symptomatic CDI was determined by a positive LAMP assay from stool of acute diarrhea

Data Sources

Retrospective Chart Review

All patients who met the inclusion criteria, and tested for *C. difficile* colonization had their electronic medical records examined to collect information on potential risk factors of interest, demographic data and presence of *C. difficile* infection as defined by the criteria above.

Abstracted Data Elements from Retrospective Chart Review

Demographic information such as patient age, sex, hospital and ward, date of admission, and discharge were extracted from the computerized hospital information system (MediTech) through MRSA/VRE test sample number. Moreover, patient history about risk factors of interest such as high-risk comorbidities, recent hospitalization, and previous history of *C. difficile* infection was extracted. Length of stay was calculated in the number of days from the date of admission to date of discharge. Lastly, information on symptomatic CDI and mortality was extracted from patient health records as well. The microbiology lab provided data on the results of *C. difficile* colonization.

Data Sample, Sample Size and Power

Based on previously published literature and preliminary results, we anticipated that 5-10% of patients with MRSA/VRE swabs would be positive for colonization of *C. difficile*. Moreover, we expected that we would collect

approximately 1000 swabs to be tested for *C. difficile* using LAMP and confirmed with PCR over the study period. We also estimated that at least 60-100 carriers of *C. difficile* would be identified. Therefore, assuming the lower end of the prevalence rate of 5%, and with being 95% certain that at least 60 patients would be colonized, a sample size of 1459 patients was needed.

Baseline Characteristics

Demographic and baseline characteristics for the study sample were examined using standard descriptive statistics. For Gaussian continuous variables, averages and standard deviations are reported, and median and interquartile ranges are reported for non-normally distributed data with independent t-tests or Wilcoxon-Mann-Whitney U tests for comparative statistics respectively. Count data and percentages are reported for categorical data, with chi-square tests being used for comparative statistics. A fisher's exact test adjustment was utilized for chi-square tests if at least one cell of a contingency table has $n < 5$ counts. Baseline admission prevalence rates for asymptomatic colonization were calculated.

ANALYSIS

Primary Analysis – Logistic Regression

A multi-level logistic regression model was conducted to examine possible risk factors for asymptomatic colonization of *C. difficile* at admission (model 1) and point prevalence screening (model 2). The data was represented with two levels consisting of hospital wards and individual patients within hospital wards. The *a priori* hypothesis for needing a hierarchical model is that patients within the same hospital ward may be more similar (clustering), and having hospital ward as the random effect and covariates as fixed effect. A single-level multiple regression model treats the units of analysis as independent observations, therefore resulting in an underestimation of the standard errors of the regression coefficients leading to an overestimation of statistical significance(52).

Both models were generated by first including all the variables of interest: Age, Gender, Previous History of CDI, Recent Hospitalization and Length of Stay (the latter only for model 2). The *a priori* hypothesis was that as subjects increase in age, with a previous history of symptomatic CDI, recent hospitalization in the last 12 months and longer lengths of stay were more likely to have or develop *C. difficile* colonization. After examination of the significance of each variable to the overall model (assessed through p values), variables that were not significantly contributing to the model (p-value > 0.10), were removed from the final model, using a backward model building approach. A larger p-value of 0.10 was used as the threshold, as we were interested in a predictive model and a larger p-value is the recommended practice to ensure that important predictors are not

missed(53). Assessment of variable importance from removed variables was computed by comparing the full and reduced models' deviance statistics and AIC values, with lower values of each being indicative of a better performing model. Odds and odds ratios were reported from variables of interest. Adjusted R^2 were used to assess model performance. The assumptions for the use of logistic regression of a linear relationship between continuous predictor variables and the logit of the outcome variable, and multicollinearity were ruled out. Logistic regression modeling assumes linearity relationship between the independent variables and the log odds, and can be assessed through the examination of scatter plots between each covariate and logit. Logistic regression also requires that the independent variables are not highly correlated with each other and therefore are providing redundant information about the response variable, or little to no multicollinearity. Multicollinearity can be assessed with a variance inflation factor (VIF), and a VIF < 5 is generally tolerated as acceptable. A *p* value of 0.05 was considered statistically significant.

Moreover, there was the possibility that patients may have repeat admissions within the study time frame, violating the independent observation assumption for logistic regression modelling. If there were very few repeat admissions (<10%), as determined by the author, these data points would count as independent observations. However, if there was a significant proportion of the data with repeat admissions, a sensitivity analysis would be conducted using a

generalized estimating equation (GEE). The GEE's objective is a statistical approach to fit a marginal model that has correlated and clustered responses on the same individual and is a widespread technique commonly used in biomedical research. Repeated ANOVA/MANOVA measures are another method of evaluating correlated or clustered responses. However, it lacks the ability to adjust for covariates – making GEE an appropriate choice. The GEE would be constructed using a logit link function as the dependent variable is binary. Details of the correlation structure and association between the dependent variable and covariates are included in an expression known as the quasi-likelihood function.

Secondary Analysis – Survival Analysis

The exposure of interest was recent hospitalization (within 12 months), age and ward. Demographic information was obtained from electronic health records. Age was grouped as a categorical variable with younger and older than 65.

Exposures of Interest

The exposure of interest was recent hospitalization (within the last12 months), age and ward. Age was grouped as a categorical variable with younger and older than 65, in order to evaluate the effects of being elderly on colonization acquisition.

Ascertainment of Outcomes

The primary endpoint was the development of asymptomatic *C. difficile* colonization. *C. difficile* colonization was defined as via the detection of toxin-specific nucleic acid using laboratory-developed loop-mediated isothermal amplification (LAMP) methods currently used by the Hamilton Regional Laboratory Medicine Program (HRLMP) Virology/Molecular Laboratory.

Covariates of Interest

The previous history of CDI, and sex were determined through a review of electronic health records from in-patient admission.

Statistical Analyses

The study's time origin was admission to any of the study wards. Patients were followed-up from admission and censored at discharge with death as a competing risk. Left censoring was present when a patient was carrier upon admission. A Kaplan-Meier analysis was conducted in order to estimate the probability of survival over time, stratified, as a function of the ward, recent hospitalization, and age differences. A multi-level Cox proportional hazard regression model was used to assess potential risk factors for asymptomatic colonization, and scaled Schoenfeld's residuals were used to assess the proportionality of hazard (PH) assumptions. The proportionality of hazards assumption is that the hazards are proportional over time or the effect of a risk

factor is constant, and is required for the appropriate use of both the log-rank test and Cox PH regression modeling (54). The *a priori* hypothesis for needing a hierarchical model is that patients within the same hospital ward may be more similar, and having hospital ward as the random effect and covariates as fixed effects may help for potential confounding. Cox proportional hazards models were used to estimate the hazard ratio of asymptomatic *C. difficile* colonization. The model was adjusted for covariates of interest including previous colonization, recent hospitalization, age and sex. In a sensitivity analysis, death from non-*C. difficile* related causes were evaluated as a competing risk. If present, patients who were re-admitted to the same hospital ward would be treated as independent observations, and robust standard errors were used to account for intra-patient correlation among repeat admissions. Log-likelihood values will be compared between the null and fitted model to assess model performance. A two-sided p-value of 0.05 was used to assess statistical significance.

All statistical analyses were performed using RStudio Version 1.1456.

PART III: RESULTS

From January to April 2018, and September 2018 to August 2019, 1470 test swabs were collected from 1056 individual patients and tested for *C. difficile* colonization using LAMP assay and PCR, and used for analysis.

Descriptive Results

From the 1056 patients in the data sample, 72 (6.8%) were identified as asymptomatic *C. difficile* carriers using both admission and point prevalence swabs (Table 1).

The median age at baseline amongst the sample was 72 years old (IQR 61.0-84.0). The median age of colonized patients was 75 years (IQR 61.0-84.0), whereas the non-colonized patient's median age was 72 years (61.0-84.0). Colonized patients were older than non-colonized patients. However, this difference was not statically significant ($p = 0.90$). From the sample, 535 (50.6%) patients were male. Of the 72 colonized patients, 42 (58.3%) were male, whereas 506/984 (51.8%) non-colonized patients were male. The difference in gender distribution amongst colonized and non-colonized patients was not statistically significant ($p = 0.25$). The largest proportion of patients were in wards F3 (35.7%) and C3 (19.4%) of The Juravinski Hospital, and Nephrology ward (12.9%) of St Joseph Healthcare Hamilton. The largest proportion of colonized patients are in

wards C3 (19.7%) and F3 (23.4%) from The Juravinski hospital and the Nephrology ward (24.6%) at St. Joseph Healthcare Hamilton. The median length of stay amongst all patients was 19 days (IQR 8.0-41.75). The median length of stay amongst colonized patients was 25 days (IQR 13.0-53.75). The median length of stay amongst non-colonized patients was 19 days (IQR 8.0 – 41.0). The difference between length of stay amongst colonized and non-colonized patients was not statistically significant (p = 0.94).

There were 45 patients with repeat admissions in the sample, resulting in 4.2% of patients having a repeat admission. From all the colonized patients in the sample, 5 (6.9%) had a repeat admission, and 40 (4.06 %) non-colonized patients had a repeated admission. The difference in repeat admission between colonized and non-colonized groups was not statistically significant (p = 0.242). Amongst the sample, 493 (46.6%) patients had been hospitalized within the last 12 months.. Forty-eight of all colonized patients (66.6%) and 445 (45.2%) of all non-colonized patients had a recent hospitalization, with the proportion of recent hospitalization between the two groups being statistically significant (OR 2.41, 95% CI 1.464-4.10).

Among the total sample, 67 patients (6.34%) have had a previous CDI infection. 14 (1.32%) of all colonized patients and 53 (5.01%) of all non-colonized

patients have had a previous case of symptomatic CDI, with the difference in proportion between the two groups being statistically significant ($p < 0.001$).

Amongst the sample, all but two colonized patients had multiple comorbidities. The most common comorbidities amongst the patients were cardiovascular disease (52%), endocrine including diabetes (41.6%), cancer (26.3%), renal system (26.3%) and gastrointestinal (19.4%). Four patients (5.55%) were missing information on comorbidities from the electronic health records.

Comparison of Testing Strategies

Upon admission, 575 MRSA/VRE swabs were collected from 548 individual patients (Table 2). Amongst the admission swabs, 19 patients were positive for colonization, with two patients having one repeat admission each, resulting in 21 positive admission swabs and a 3.69% colonization rate on admission. Eight hundred ninety-four point prevalence swabs were collected from 615 individual patients, and 51 patients were found to be positive for asymptomatic *C. difficile* colonization, resulting in a prevalence rate of 8.26% for point prevalence testing.

Twelve (16%) patients were negative on admission and later found to be positive for colonization through point prevalence testing. Another twelve patients

had tested negative during point prevalence testing and then were found to be positive during subsequent point prevalence tests; however, they were missing admission testing results. In total, 24/51 patients (33%) had developed *C. difficile* colonization during their hospital stay. Twenty-seven (33.3%) patients had a positive point prevalence test for colonization during hospital admission; however, they were missing test results on admission as well as subsequent point prevalence results throughout their stay; therefore, the natural history of colonization could not be assed amongst this patient group.

When comparing testing methods (admission vs. point prevalence) in identifying asymptomatic *C. difficile* carriers, the difference in prevalence estimates between the two groups is non-significant (OR 0.561, 95% CI 0.016, 7.42). In comparison, those 24 patients who developed *C. difficile* colonization during their hospital stay had a median age of 72.50 years (60.75 – 84.50), whereas those 12 patients who had *C. difficile* colonization on admission had a median age of 70 years (58.75-82.25) (Table 3). Sixteen (72.7%) of the 21 patients positive for *C. difficile* colonization on admission were male, whereas 15 (62.5%) patients were male of those who developed *C. difficile* colonization throughout their hospital stay. Six (28.5%) patients who were positive on admission has a previous case of symptomatic CDI, whereas 1 (4.16%) patient who developed *C. difficile* colonization during their hospital stay had a previous case of CDI. Eleven (52.3%) patients who were positive for *C. difficile*

colonization had a recent hospitalization within the last 12 months, 11 (45.8%) patients who developed *C. difficile* during their hospital stay also had a recent hospitalization within the last 12 months. The differences between characteristics were not significant across both groups. There was no significant difference in comorbidities between both groups.

Risk Factors for Colonization, Upon Admission (n = 21)

Risk factors for *C. difficile* colonization at admission are outlined in Table 4. Using a multivariable hierarchical logistic regression model, patients who were colonized on admission (n = 21) had previous CDI ($p < 0.001$) and female ($p< 0.05$) as statistically significant for covariates for colonization. Those with previous CDI were 4.76 times more likely to be colonized at admission (OR 4.76, 95% CI 1.49-13.86). Being female was associated with a 2.85 times higher odds of being colonized at admission compared to males (OR 2.66, 95% CI 1.02-8.33).

Assumption Testing and Model Fit

The full model with all predictors (age, sex, previous CDI, recent hospitalization) had an AIC value of 152.8, and Deviance Residuals of 140.8. The R^2 of the full model was 30.0% indicating moderate to-poor predictive probability. The final model with predictors sex and previous CDI were retained after backwards model selection had an AIC of 150.9 and deviance residual value of 140.9, and an adjusted R^2 value of 30.15% indicating moderate-to-poor predictive

probability. The similarity of the AIC scores indicate that their model fit performances are not well differentiated from each other. The final model performed marginally better than the full model. All assumptions were met in order to perform a logistic regression model.

Risk Factors for Colonization, Point Prevalence (n = 24)

Risk factors for acquisition of *C. difficile* colonization during hospitalization are outlined in Table 5. Using a multivariable hierarchical logistic regression model, patients who were colonized throughout hospitalization (n = 24) were compared to patients who were not colonized throughout hospitalization (n = 229). Patients who were not colonized throughout hospitalization included patients who had available negative admission and subsequent point prevalence swabs or, a series of negative point prevalence swabs in the absence of an admission swab. When patients who were colonized throughout a hospital stay were compared to patients who were not colonized throughout their hospital stay, previous CDI ($p < 0.001$) and recent hospitalization within the last 12 months ($p < 0.001$), were significant predictors. Patients with previous CDI were associated with a 4.77 higher odds to be colonized with *C. difficile* throughout their hospital stay (OR 4.77, 95% CI 2.15-9.97). Patients with a recent hospitalization were 2.43 times more likely to be colonized with *C. difficile* than those without a recent hospitalization (OR 2.43, 95% CI 1.29-4.40).

Assumption Testing and Model Fit

The full model with all predictors (Age, sex, previous CDI, recent hospitalization, length of stay) had an AIC value of 398.7, and Deviance Residuals of 384.7. The R^2 of the full model was 11.86% indicating poor predictive probability. The final model with predictors sex, recent hospitalization and previous CDI after backwards model selection had an AIC of 394.8 and deviance residual value of 384.8, and an adjusted R^2 value of 12% indicating poor predictive probability. The similarity of the AIC scores indicate that their model fit performances are not well differentiated from each other. The final model performed marginally better than the full model. All assumptions were met in order to conduct a logistic regression model.

Time-to-Colonization of *C. difficile* (n = 24)

Using a multi-level Cox PH model, patients who became colonized during their hospitalization (n = 24) were compared to patients who were not colonized throughout hospitalization (n = 229) (Table 6). Patients missing admission and subsequent point prevalence swabs were excluded from the analysis. The Kaplan-Meier survival curve for time to colonization of asymptomatic *C. difficile* stratified by recent hospitalization demonstrated that patients with a recent hospitalization on average had a greater proportion of patients developing asymptomatic colonization in comparison to those who did not have a recent admission over time. The difference between both survival estimates is

statistically significant, as determined by the log-rank test ($p < 0.001$). The Kaplan-Meier survival curve for time to the colonization of asymptomatic *C. difficile* stratified by Ward demonstrated that the Hemodialysis Outpatient ward (ward 9) stood out with a greater fraction of patients developing asymptomatic colonization over time. The CTU Central Ward (Ward 8) and 8S (Ward 1) had the lowest proportion of patients developing asymptomatic colonization over time. The difference between rates of asymptomatic colonization by ward was statistically significant ($p < 0.001$). The Kaplan-Meier survival curve showed that those younger than 65 years had a larger proportion of patients developing asymptomatic colonization over time in comparison to those older than 65 years. As determined by the log-rank test, the difference between the two groups were not statistically significant ($p = 0.38$).

Using a multilevel Cox proportional hazards model, with ward being the random effect and recent hospitalization, age, being female and previous CDI being the fixed effects, both recent hospitalization ($p < 0.001$) and previous CDI ($p <0.001$) were significant predictors. Being recently hospitalized in the past 12 months was associated with a 2 times increase in the hazard of asymptomatic colonization (HR 2.21, 95%CI 1.320,3.731). Having previous CDI was associated with a 2.29 times increase in the hazard of asymptomatic colonization (HR 2.40, 95% CI 1.340,4.305). The proportional hazards assumption was verified using scaled Schoenfeld's residuals, and none of the covariates or global measures

were significant, indicating that the proportionality of hazards assumption was satisfied.

Progression to CDI

From the 72 asymptomatic colonized patients in the sample, 14 went on to develop symptomatic CDI infection (19.44%). From the 21 patients who were positive on admission, 5 (23.8%) developed symptomatic CDI while 16 remained colonized with no symptomatic CDI. From the point prevalence group, 7 went on to develop symptomatic CDI while 17 patients (29.1%) remained colonized with no symptomatic CDI (Figure 1). There were no difference in characteristics between the symptomatic CDI developed from those who were colonized and those who remained colonized with no CDI.

TABLE 1 – Baseline Characteristics of Adult Inpatients Admitted to The Juravinski, Hamilton General or St Joseph's Healthcare Hamilton Hospitals

	Overall n = 1056	**Colonized n = 72**	**Not Colonized N = 984**	***p*-value* *(Comparing Colonized .vs Not Colonized)***
Age at baseline in years (median, IQR)	72 (61.0, 84.0)	75 (61.0,84.0)	72 (61.0,84.0)	0.9
Sex, Male (%)	535 (50.6)	42 (58.3)	483 (49.0)	0.25
Ward (%)				
Juravinski				
- C3	205 (19.4)	16 (19.7)	189 (19.2)	0.53
- C4	34 (3.21)	1 (1.23)	33 (3.35)	0.36
- E3	66 (6.24)	6 (7.40)	60 (6.09)	0.44
- F3	378 (35.7)	19 (23.4)	359 (36.4)	0.08
Hamilton General Hospital	81 (7.66)	2 (2.46)	79 (8.02)	0.10
- 8S	44 (4.16)	5 (6.17)	39 (3.96)	0.22
- 8W				
St Joseph Hamilton Healthcare	137 (12.9)	20 (24.6)	117 (11.8)	<0.001 **
- Nephrology	59 (5.58)	0 (0)	59 (5.99)	0.03 **
- CTU(Central)	61 (5.77)	3 (3.70)	58 (5.89)	0.56
- Hemo (Outpatient)				
Repeat Admissions (%)	45 (4.26)	5(6.94)	40 (4.06)	0.242
Recent Hospitalization*	493 (46.6)	48 (66.6)	445(45.2)	<0.001**

(within the last 12 months) (%)				
Previous CDI (%)	67 (6.34)	14(1.32)	53 (5.01)	<0.001 **
Length of Stay in days (median, IQR)	19 (8.0, 41.75)	25 (13, 53.75)	19 (8, 41.00)	0.94
Comorbidities - Cardiovascular - Gastrointestinal - Endocrine (including Diabetes) - Immune - Cancer - Neurologic - Renal System - Respiratory - Bone - Other No Comorbidities Missing	-	 52 (72.2) 14 (19.4) 30 (41.6) 8 (11.1) 19 (26.3) 9 (1.25) 19 (26.3) 13 (18.0) 14 (19.4) 10 (13.8) 2 (2.77) 4 (5.55)	-	-

* For Gaussian continuous variables, averages and standard deviations are reported, and median and interquartile ranges are reported for non-normally distributed data with independent t t-tests or Wilcoxon-Mann-Whitney U tests for comparative statistics respectively. Count data and percentages are reported for categorical data, with chi-square tests being used for comparative statistics

TABLE 2a – Comparison of *C. difficile* Screening Identification Methods of High-Risk MRSA/VRE In-patients

	Overall	Positive for Colonization *	Prevalence Rate
Admission Swabs (n of Patients)	548	21	3.69%
Point Prevalence Swabs (n of Patients)	617	55	8.26%
Patients (n) with Both Admission and Point Prevalence Swabs	107	12	11.12%
Patients (n) with Repeated Point Prevalence (> 1) Tests	171	52	30.4%
Overall	1056	72	6.81%

* As determined through LAMP Assay and confirmed with PCR

TABLE 2b – Comparison of *C. difficile* Screening Identification Methods of High-Risk MRSA/VRE In-patients

	n of Patients	OR	95% CI (lower, upper)	p-value ***
Patients who were positive on admission (n = 548)	21	2.37	(1.29,4.34)	< 0.001
Patients who developed *C. difficile* during hospital stay** (n = 278)	24			
Patients missing admission and subsequent point prevalence swabs	24	-	-	-

*Includes patients who we had evaluable data for. Was calculated using those positive for colonization out of all admission swabs in the numerator. In the denominator, includes those who were followed up and positive for colonization, in comparison to those followed up and negative for admission.
**includes those who had negative admission tests, and had positive point prevalence swabs, and those who had negative point prevalence swabs, and later had positive point prevalence swabs but were missing an initial admission swab
** For gaussian continuous variables, averages and standard deviations are reported, and median and interquartile ranges are reported for non-normally distributed data with independent t t-tests or Wilcoxon-Mann-Whitney U tests for comparative statistics respectively. Count data and percentages are reported for categorical data, with chi-square tests being used for comparative statistics

TABLE 3 – Comparison of Patients who are Positive at Admission and Positive during Hospital Stay

	Positive for *C. difficile* at admission n = 21	**Negative for *C. difficile* at admission, positive at point prevalence testing n = 24**	***p*-value** *
Age in years	70 (58.75 – 82.25)	72.50 (60.75 – 84.50)	0.40
Sex (% male)	16 (76.1)	15 (65.2)	0.32
Previous CDI (%)	6 (28.5)	1 (4.16)	0.06
Recent Hospitalization (%)	11 (52.3)	11(45.8)	0.77
Comorbidities (%)			
- Cardiovascular	8 (38)	15 (62.5)	0.57
- Gastrointestinal	2 (9.5)	5 (20.8)	0.46
- Endocrine (including Diabetes)	11(52.3)	11 (45.8)	0.38
- Immune	2 (9.5)	2 (8.33)	0.73
- Cancer	3 (14.2)	9 (37.5)	0.21
- Neurologic	2 (9.5)	4 (19.0)	0.66
- Renal System	7 (33.3)	10 (41.6)	0.95
- Respiratory	3 (14.2)	5 (20.8)	0.80
- Bone	4 (19.0)	4 (16.6)	0.62
- Other	2 (9.5)	2 (8.33)	0.73
No Comorbidities	1 (4.76)	0 (0)	0.23
Missing	3 (14.2)	0 (0)	

* For gaussian continuous variables, averages and standard deviations are reported, and median and interquartile ranges are reported for non-normally distributed data with independent t t-tests or Wilcoxon-Mann-Whitney U tests for

comparative statistics respectively. Count data and percentages are reported for categorical data, with chi-square tests being used for comparative statistics

TABLE 4 – Predictors of *C. difficile* Colonization and Model Fit of Multivariable Analysis (Admission)

	Multivariable Analysis (Model 1, Admission, Full Model)			**Multivariable Analysis (Model 1, Admission, Final Model)**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at baseline	1.00	0.97,1.04	0.801	-	-	-
Sex (Female)	2.85	1.00,9.09	**0.06**	2.66	1.02,8.33	**0.05**
Previous CDI	3.20	0.88,10.29	**0.05**	4.76	1.49, 13.86	**<0.001**
Recent Hospitalization	1.85	0.66,5.67	0.247	-	-	-
Length of Stay	-	-	-	-	-	-
AIC/Deviance Residuals	AIC: 152.8 Deviance: 140.8			AIC: 150.9 Deviance Residuals: 140.9		
R^2	Adjusted R^2 = 30.0%			Adjusted R^2 = 30.15%		

TABLE 5 – Predictors of *C. difficile* Colonization and Model Fit of Multivariable Analysis (Point Prevalence)

	Multivariable Analysis (Model 1, Admission, Full Model)			**Multivariable Analysis (Model 1, Admission, Final Model)**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at baseline	1.00	0.98-1.02	0.85	-	-	-
Sex (Female)	1.06	0.59,1.92	0.84	-	-	**-**
Previous CDI	4.77	2.15,9.97	**<0.001**	4.75	2.14,9.94	**<0.001**
Recent Hospitalization	2.43	1.29,4.40	**<0.001**	2.35	1.30,4.42	**<0.001**
Length of Stay	0.99	0.99-1.00	0.28			
AIC/Deviance Residuals R^2	AIC: 221.6 Deviance: 207.6 Adjusted R^2 = 13.94%			AIC: 216.71 Deviance Residuals: 208.7 Adjusted R^2 = 14%		

TABLE 6 – Results of Cox mixed-effects model on time-to-colonization of Asymptomatic *C. difficile* Colonization

	HR	95% CI		p-value
		Lower	Upper	
Age	1.00	0.982	1.019	0.750
Sex(Female)	1.25	0.77	1.99	0.650
Recent Hospitalization	2.21	1.320	3.731	**0.001** **
Previous CDI	2.40	1.340	4.305	**0.001** **
	Null Log-likelihood: -443.2568 Fitted Log-likelihood: -426.016			

TABLE 7 – Comparison of *C. difficile* Carriage Progression to Symptomatic CDI

	Asymptomatic *C. difficile* Colonization, with Progression to Symptomatic CDI (n = 14)	**Asymptomatic *C. difficile* Colonization, With no Progression to Symptomatic CDI (n = 58)**	**OR**	**95%CI (lower,upper)**	**p-values**
Age	67.0 (54.0-74.50)	76 (61.25-85.0)	0.95	0.73,1.17	0.61
Sex (% male)	9 (64.2)	37 (63.7)	1.00	0.316,3.412	0.97
Comorbidities (%)					
- Cardiovascular	4 (28.5)	48 (82.7)	0.08	0.02,0.32	**< 0.001**
- Gastrointestinal	2 (14.2)	12 (20.6)	0.64	0.13,3.25	0.58
- Endocrine (including Diabetes)	6 (42.8)	24 (41.3)	1.06	033,3.46	0.91 0.17
- Immune	3 (21.4)	5 (8.62)	2.89	0.60,13.92	0.25
- Cancer	2 (14.2)	17 (29.3)	0.40	0.08,1.99	0.82
- Neurologic	2 (14.2)	7 (12.0)	1.21	0.22,6.60	0.63
- Renal System	3 (21.4)	16 (27.5)	0.72	0.18,2.90	0.68
- Respiratory	2 (14.2)	11 (18.96)	0.71	0.14,3.65	0.19
- Bone	1 (7.1)	13 (22.4)	0.27	0.03,2.23	0.41
- Other	1 (7.1)	9 (15.5)	0.42	0.05,3.61	0.48
No Comorbidities	0 (0.0)	2 (3.44)	-		0.312

Missing	0 (0.0)	4 (6.89)	-		
Previous CDI (%)	5 (35.7)	13 (22.4)	2.51	2.11,2.91	0.30
Recent Hospitalization (%)	11 (78.5)	44 (75.8)	1.10	0.320,4.55	0.83

FIGURE 1 – Retrospective Cohort Study, in-Patient progression

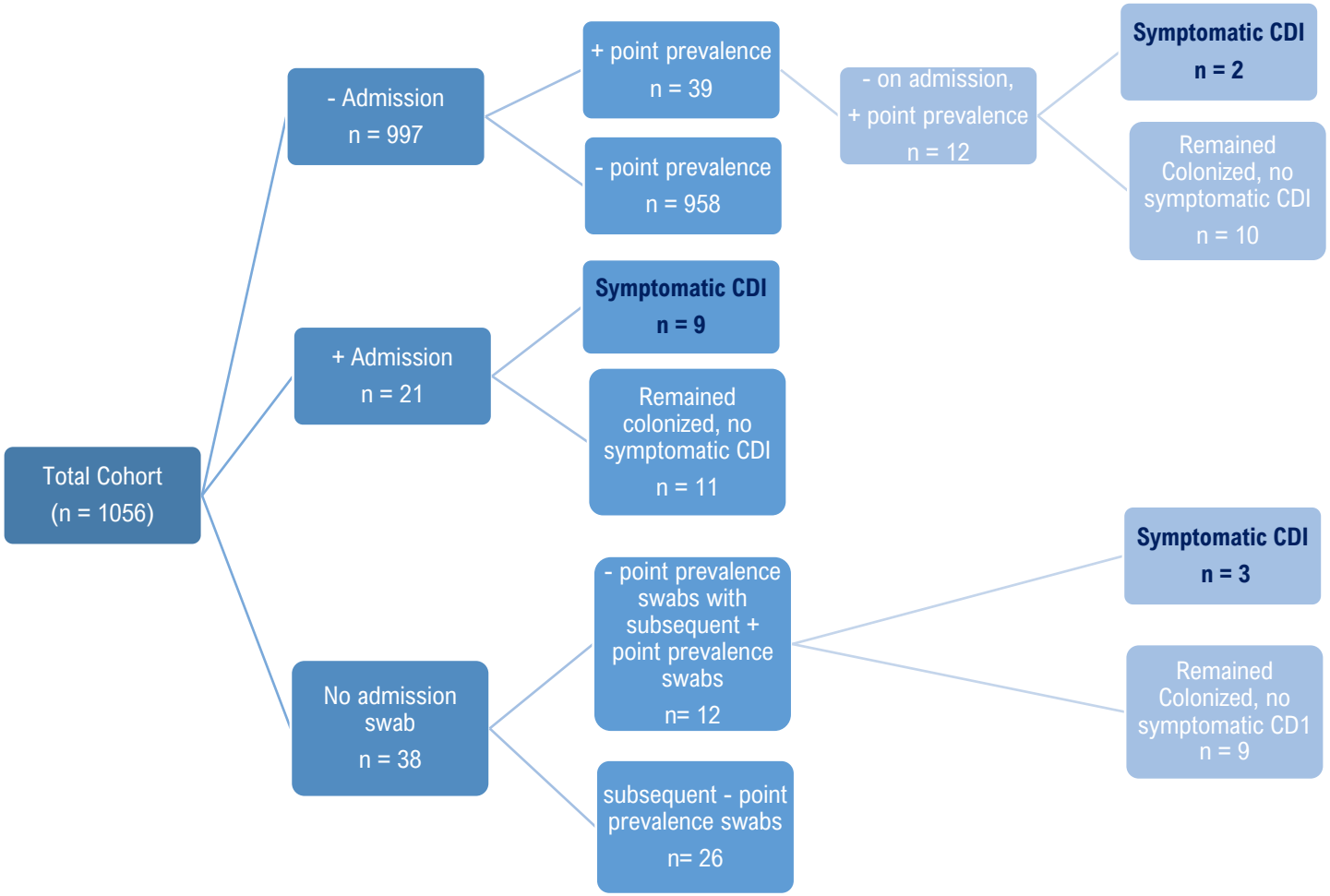


FIGURE 2 – Kaplan Meier Estimates for Time to Colonization during Hospitalization of Asymptomatic *C. difficile* stratified by Recent Hospitalization

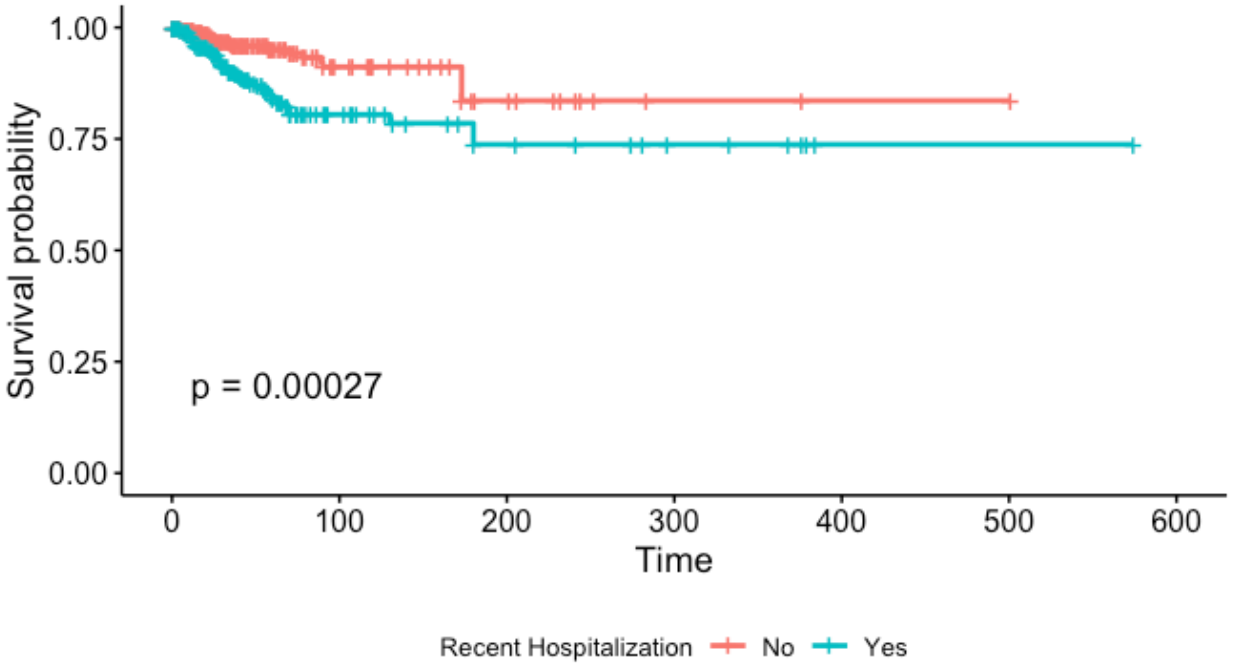


FIGURE 3 – Kaplan Meier Estimates for Time to Colonization during Hospitalization of Asymptomatic *C. difficile* stratified by Ward

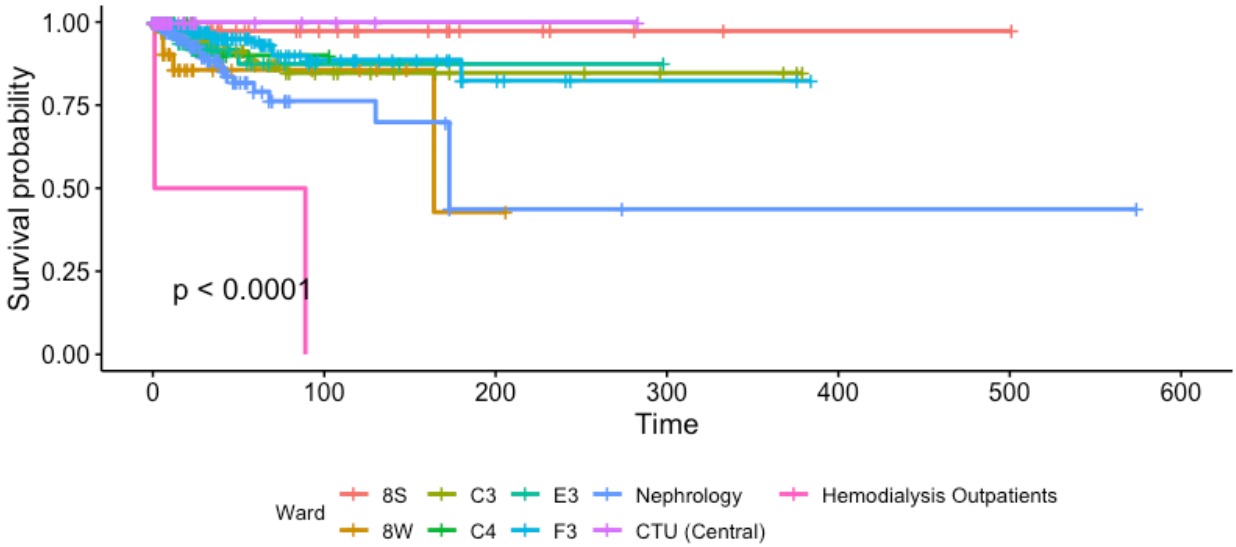
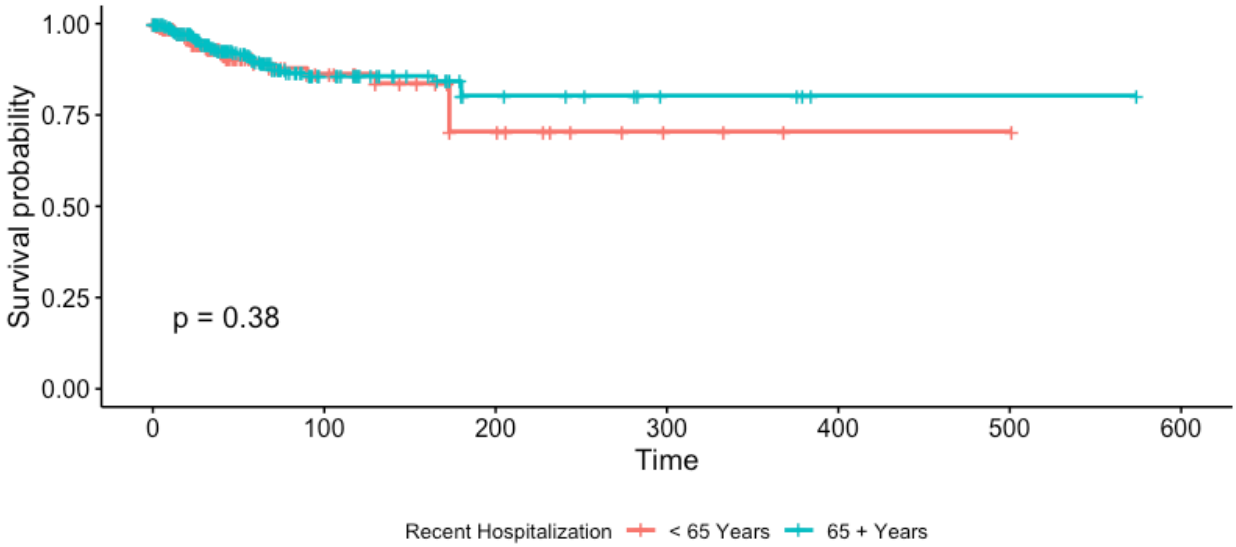


FIGURE 4 – Kaplan Meier Estimates for Time to Colonization during Hospitalization of Asymptomatic *C. difficile* stratified by Age Categories younger and older than 65 years



PART IV: DISCUSSION

Summary of Findings

Our retrospective cohort study included 1056 individual patients admitted to 9 inpatient wards over 15 months, with 72 positive LAMP tests for asymptomatic colonization, resulting in an overall prevalence rate of 6.81% (range 0% - 24.6%). There were no cases of asymptomatic colonization on the CTU (Central) ward in SJHH. The Nephrology ward in SJHH had the largest percentage (24.6%) of patients who were asymptomatically colonized throughout the study period. Twenty-one (3.69%) of patients with admission swabs were colonized with *C. difficile*, and 24 (8.63%) patients became colonized during their hospital stay. Twenty-four patients had positive point prevalence swabs, however with missing admission swabs or subsequent point prevalence swabs, therefore establishing the natural history of colonization amongst this group was not possible. In comparing testing strategies, there were no significant differences in the identification of inpatients; however, point prevalence testing identified an additional 51 cases and a testing strategy that involves both point prevalence, and admission screening is imperative. Risk factors for colonization at admission were and previous CDI, while risk factors for colonization at point prevalence were previous CDI and recent hospitalization. Those with a recent hospitalization and from Hemodialysis and Nephrology wards had the most significant proportion of patients who progressed to asymptomatic colonization over time. Recent

Hospitalization and previous CDI were also significant predictors in the hazard rate of *C. difficile* colonization. Fourteen colonized patients developed symptomatic CDI; however, there were not significant differences in sex, age, previous CDI, previous hospitalization and comorbidities, except for cardiovascular diseases between those who developed symptomatic CDI and those who remained asymptomatic and colonized.

Prevalence of Asymptomatic *C. difficile* Colonization

Over the collection period of January to April 2018 and September 2018 to April 2019, *C.*
difficile colonization prevalence across a mixed adult inpatient population was 6.81%. Compared to other studies identified in the narrative review, the colonization rate of 6.81% falls within the lower end of the reported patient colonization rate of 4-29%(11, 36). Higher estimates of colonization rates in studies have been due to differences in a patient population, study location, testing strategies and laboratory methods. The prevalence rate on admission was 3.69%, consistent with reported admission colonization rates of other published literature. For example, general internal medicine or mixed wards with adult inpatients in the United States, reported prevalence rates for asymptomatic carriage are in the range of 4.4-15.4%. Two larger-scale studies by Kong et al.

and Longtin et al. had a prevalence rate of 4.05% and 4.8%, respectively(36, 42). However, it is relevant to note that both of the studies, as mentioned earlier, had a larger sampled population size (7000-9000 sampled patients) and may suggest why colonization rates for these studies were slightly higher as our study did not meet sample size recruitment necessary to detect our desired prevalence rate. The prevalence rate for colonization amongst point prevalence testing strategy was 8.26%. Studies of prevalence rates of hospitalized patients have been shown to range from 3-21%. Literature has suggested a 50% acquisition rate for patients with a length of stay greater than four weeks for asymptomatic *C. difficile* colonization. The failure to reach adequate sample size by 403 patients may indicate why our prevalence estimate was on the lower end of the scale.

Twenty-one patients were identified on admission as positive for colonization, while point prevalence swabs identified 24 patients who developed colonization throughout their hospital stay, identifying an additional 54% of carriers. Therefore, a joint strategy of identifying patients on admission in addition to point prevalence screening is ideal. This finding is consistent with the literature. One study had found an admission prevalence rate of 9.5%, and weekly testing revealed an additional 7.2% of patients that acquired carriage after admission. Another study found an increase of 50% in colonization rates over a hospital stay despite a low (2.1%) admission rate. The difference between in-patients screened on admission and those with point-prevalence screenings were

statistically significant ($p = 0.001$) in carriage identification, with universal point prevalence screening identifying 2.37 times more colonization patients than admission screening along (OR 2.37, 95% CI 1.29, 4.34) suggesting that the addition of universal point prevalence screening in addition to admission screening helped identify more than double the amount of carriers in the population.

Risk Factors for Asymptomatic Colonization, at admission and point prevalence

Our study identified that being female and previous CDI were risk factors for *C. difficile* colonization on admission. Recent hospitalization and previous CDI were risk factors for *C. difficile* colonization throughout a hospital stay. These risk factors are consistent with our findings from the narrative review for the majority. One study had found that previous CDI and recent hospital stay were significant predictors for colonization on admission, and the use of proton pump inhibitors, corticosteroids, and antibiotic use before admission were not significant predictors. Our study agrees with these results; however, data on medication history was not collected and suggest future research steps and a limitation of the study.

Moreover, literature has also suggested that risk factors for developing *C. difficile* during hospital stay were previous hospitalization, proton pump inhibitors, H2 blockers, chemotherapy, previous CDI, an increased number of comorbidities. Our findings are somewhat consistent with the literature, as we identified previous hospitalization and previous CDI as risk factors of importance. Still, as previously mentioned, an exploration of medication therapy and comorbidities were not analyzed in this study. Comorbidities amongst patients, especially those known to be high risks such as cancer and gastrointestinal diseases and medication therapy would be useful for further research, and could improve the predictive probability of the regression model. The large range of 95% confidence intervals from parameter estimates are indicative of the analysis being underpowered, due to an inadequate sample size.

Our study sample had a repeat admission of less than 10%; therefore, a GEE correction was not used.

Time-to-Colonization

The Kaplan-Meier estimates revealed that patients with a recent hospitalization, and being admitted to hematology and nephrology wards were at higher risk for developing asymptomatic colonization over time. This is consistent with the literature, as recent admission is consistently an identified risk factors for *C.*

difficile colonization development. Moreover, studies have identified comorbidities of high risk for *C. difficile* colonization to include renal-related comorbidities and immunosuppressed patients, such as those diagnosed with hematologic cancers, which are typical patients on both Nephrology and Hematology wards(47). Moreover, the multilevel cox proportional hazard model identified previous CDI and recent hospitalization to be risk factors for developing *C. difficile* colonization throughout a hospital admission. These results reiterate what has been found in the logistic regression models and solidify what had been identified in the literature.

Progression to CDI

The role of *C. difficile* colonization and the development of symptomatic CDI is not well understood. 19.4% (14/72) of colonized patients in the study went on to develop symptomatic CDI. Our prevalence of progression to symptomatic CDI is consistent with the literature. In a published systematic review, 21.8% (95% CI 7.9-40.1%) of colonized adult inpatients on admission developed a subsequent CDI episode during their hospital stay. However, another study reported rates as low as 2.5% in progression to symptomatic CDI, a mixed lower-risk patient population. The significant heterogeneity in the progression results to symptomatic CDI from asymptomatic colonization can vary from reason such as strain, testing methods, and other

patient population differences. Age, sex, previous CDI, recent hospitalization, and comorbidities except for cardiovascular diseases (OR 0.08, 95% CI 0.02-0.32) were not risk factors in the development of symptomatic CDI amongst those already asymptomatically colonized, with cardiovascular disease being protective of symptomatic CDI development.

Limitations

One of the limitations of this study was the processes in data collection. In the beginning stages, swabs were supposed to be marked with a sticker by the nurse on the ward, and when received at the microbiology lab forwarded to the research laboratory for storage until testing may need to be required. However, some of the labels were not sticking to the physical tubes of samples, and often, the nursing staff forgot to attach stickers. Furthermore, stickered specimens may not have been reliable put aside by the laboratory personnel. Thus swabs were getting thrown away as they were thought to not be from wards of interest and resulted in thinking that there was an under-collection of swabs from wards of interest. It wasn't for several months until the lab-processed could get changed to have all swabs reliably collected, and individually screened by hand to make sure they were from wards of interest. This new strategy was more labour intensive but ensured that no specimens went missing. However, the lack of a reliable process prior to implementation of this new process resulted in missing

specimens and in a segmented collection date with no swabs collected between April 2018 and August 2018. Moreover, due to the global pandemic of COVID-19 being announced in early March, laboratory operations and *C. difficile* colonization testing were not available to add more samples to the patient population, whether it was more colonized inpatients or more point prevalence swabs of already admitted patients. Therefore, the sample size was not met by 403 patients. Moreover, not all patients had available admission and subsequent point prevalence swabs with only 24 out of 51 point prevalence swabs available for evaluation. The limitations in not achieving sample size, may result in models being statistically underpowered to detect a significant difference.

Another limitation of the dataset is due to secondary data review. Because there was no primary data collection in the study, information was extracted from electronic health records. However, there was inconsistent information on variables such as admission and discharge date, and clarity in medication therapies and current comorbidities, which would have been useful information for the nature of this study.

In addition to the limitations, as mentioned above, predictors were chosen based on the literature. Age and gender were accurately captured, but if CDI occurred at another facility outside of HHS; there was no information on the occurrence, the same for recent hospitalization. Moreover, recent hospitalization

was defined as a hospitalization within 12 months prior to admission, and therefore the impact of the duration (i.e. long vs short hospital stay) of the previous hospitalization was not assessed.

PART V: CONCLUSION & FUTURE STEPS

In conclusion, the results of this study add significantly to the existing literature currently on *C. difficile* colonization. The overall admission and point prevalence colonization prevalence rates add to the current estimates of *C. difficile* colonization. Moreover, the review of different testing strategies show that a mixed procedure involving both admission and point prevalence testing is essential in order to identify the majority of patients colonized with *C. difficile*. Our risk factors identified on admission for *C. difficile* colonization are being female and having recent hospitalization. Our risk factors identified during hospitalization for colonization were previous CDI and recent hospitalization. Our survival analysis model that demonstrated that high-risk wards including hemodialysis and nephrology can inform health care providers to which patients may be high risk to be carriers for *C. difficile* colonization. Lastly, our study on progression to CDI identified that approximately 20% of colonized patients would progress to symptomatic CDI throughout their hospital stay which can be informative for decision making in the future.

Future steps for this research would be to collect data on comorbidities and antibiotic therapy to assess these variables in the natural history of *C. difficile* colonization. Moreover, this study was conducted in three acute care sites across Hamilton, Ontario. A more diverse study sample including multiple cities would

help make the results of this study more applicable across Ontario. Due to issues in consistent data collection over a period of time, a future step would be to obtain data from a more consistent frame in order to study potential seasonality of colonization acquisition. Lastly, in order to truly assess the role of asymptomatic *C. difficile* carriers in the transmission and contribution to hospital-wide outbreaks, epidemiologic links between cases should be established. Whole-genome sequencing and contact tracing methods in order to determine what proportion of those with symptomatic CDI develop disease resulting from their colonizing strain acquired before admission to the hospital would be a direction of further investigation.

At this time, our study adds to the existing literature testing strategies and risk factors for *C. difficile* colonization, and progression to CDI amongst colonized patients.

APPENDIX

Site	Colour	Ward	Number
Hamilton General Hospital (HGH)	Pink	8S	1
		8W	2
Juravinski Hospital	Yellow	C3	3
		C4	4
		E3	5
		F3	6
St. Joseph's Healthcare Hamilton (SJHH)	Green	Nephrology	7
		CTU (Central)	8
		Hemodialysis Outpatients	9

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