CLOSTRIDIUM DIFFICILE IN STOOL SAMPLES IN AN ACADEMIC HOSPITAL'S INTENSIVE CARE AND HIGH CARE UNIT

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Declaration

I, Amorie Botha, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Anaesthesiology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Signed

On this day of 2018

Abstract

Clostridium difficile is the most common causal pathogen for both antibiotic associated and nosocomial infectious diarrhoea. *Clostridium difficile* infection (CDI) in the intensive care unit (ICU) contributes to higher mortality and increased length of hospital stay. Institutional knowledge regarding CDI occurrence and management has the potential to improve management of CDI.

The aim of this study was to determine the positive yield and number of NAP1 strains of all stool samples sent for *Clostridium difficile* testing from 576 ICU and 579 high care unit (HCU) at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from 1 January 2014 until 31 December 2015.

A retrospective, descriptive, contextual design was followed in this study. Data from the National Health Laboratory Services (NHLS) and patients' clinical records were reviewed.

A total number of 283 samples from 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015 were included in this study. Of these samples 38 (13.42%) tested positive for *Clostridium difficile.* Out of the total number of 3 941patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015, 38 (0.96%) samples from 26 individual patients (0.66%) sent for *Clostridium difficile* tests were positive.

The median time from onset of diarrhoea until a stool sample was received in the laboratory was two days. The median time until reported diagnosis for *Clostridium difficile* polymerase chain reaction (PCR) was five hours and 23 hours for *Clostridium difficile* toxin enzyme immunoassay (EIA) test. The time difference was significant. The median time from onset of diarrhoea until treatment was initiated was 40 hours. In this study 24 patients (20%) received empiric treatment. Of the 43 patients who were started on treatment 28 (65.12%) were started on both oral and intravenous metronidazole. The median APACHE II score of 22 for CDI positive patients was significantly higher compared to the median APACHE II score of three for CDI negative patients. The APACHE II scores for patients who tested positive for *Clostridium difficile* were significantly higher than those who tested negative ($p < 0.0001$).

This study concluded that 13.42% of samples sent for CDI tests were positive and the CDI positive patients had significantly higher APACHE II scores. A significant turnaround time

difference between the *Clostridium difficile* PCR and EIA was also demonstrated. Institutional knowledge of the burden of disease of CDI as well as current practice can aid in better clinical decision making and improve patient outcome.

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List of abbreviations

CCDI	Complicated *Clostridium difficile* infection
CCNA	Cell culture neutralization assays
CDI	Clostridium difficile infection
CDBT	Clostridium difficile binary toxin
CT	Computed tomography
EIA	Enzyme immunoassay
FDA	Food and Drug Administration
FMT	Faecal microbiota transplant
ICU	Intensive care unit
HCU	High care unit
GDH	Glutamate dehydrogenase
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Services
PCR	Polymerase chain reaction
RCDI	Recurrent *Clostridium difficile* infection
RNA	Ribonucleic acid

Chapter 1: Overview of the study

1.1 Introduction

In this chapter a brief overview of the background to the study, problem statement, aims and objectives, research assumptions, demarcation of the study field, ethical considerations, research methodology, significance, validity and reliability, and the study outline will be discussed.

1.2 Background

Clostridium difficile is an anaerobic, gram positive bacterium that produces both spores and toxins (1). In 1978 Bartlett et al (2) determined that *Clostridium difficile* was the causal agent of almost all cases of pseudomembranous colitis and 20% of uncomplicated antibiotic associated diarrhoea. *Clostridium difficile* is not only the leading causal pathogen of antibiotic associated diarrhoea (3) but also the most common causal pathogen for nosocomial infectious diarrhoea (4, 5). Whilst most nosocomial infections have declined since 2001 the incidence of *Clostridium difficile* infection (CDI) has increased (1, 6). The increased incidence can be attributed to the widespread use of broad spectrum antibiotics (3, 7). In several hospitals CDI has surpassed methicillin resistant *Staphylococcus aureus* as the most common cause of nosocomial infection (4).

In recent years, *Clostridium difficile* has gained some notoriety following the emergence of the North American pulsed field gel electrophoresis type 1 (NAP1) strain (8). NAP1/BI/027 is responsible for the dramatic increase in the incidence of CDI in Europe and North America since 2000 (1, 9, 10) as well as the dramatic increase in mortality and morbidity associated with CDI (5, 10) . This strain is more likely to be resistant to treatment and has an increased frequency of relapse (5).

Data regarding CDI as well as the incidence of the NAP1 strain in South Africa is limited, with only a handful of studies published. A prospective study done at Groote Schuur Hospital by Rajabally et al (11) over a period of 15 months found CDI in 9,2 % of patients who either

presented to hospital with diarrhoea or developed diarrhoea during their admission. The incidence of nosocomial CDI was 0,087% of admissions (11). Out of the 643 patients included in this study only two cases of the NAP1 strain were reported (11). The incidence of nosocomial CDI is 0,953% in the United States (5).

It appears that the incidence of CDI is lower in South Africa than reported in the United States, but there is a need for further investigation in South Africa when one considers the paucity of South African literature.

Diarrhoea in the intensive care unit (ICU) is a common occurrence with prevalence ranging from 8-38% (12-14). Patients in ICU are very susceptible to diarrhoea due to the fact that there are multiple factors that reduce their immune response and aggravate diarrhoea (13). Diarrhoea is independently associated with increased length of stay and increased mortality (18).

The incidence of CDI in the ICU population is significantly higher, nearly double that of the general hospital population (14). Not only are ICU patients at increased risk for contracting CDI but also have higher rates of developing severe or complicated disease that result in longer length of stay. Increased length of stay especially in ICU adds to the financial burden and negatively impacts resources in the health care system. This is especially true in a country such as South Africa where specialised services such as critical care are extremely limited. CDI in ICU has far reaching implications on both mortality and morbidity. The mortality rate for ICU patients with CDI infection were found to be as high as 32%, which is significantly higher than for those without CDI (14). A review of the local literature yielded no studies that pertained specifically to *Clostridium difficile* in the ICU population.

Metronidazole and vancomycin are both effective treatments in proven cases of CDI the evidence in the literature regarding the benefit of empiric treatment however are scarce. A single study published in 2003 by Vasa et al (15) on the effectiveness of empiric metronidazole for presumed CDI found that patients who were negative for *Clostridium difficile* that were treated empirically received no clinical benefit. It did however show that patients who indeed had CDI benefited from empiric therapy (15). They showed a significantly faster resolution of symptoms, 3 days compared to 4.2 days in CDI negative patients (15). The authors recommended that empiric treatment be reserved for high risk patients who were unable to tolerate diarrhoea (15).

In view of the aforementioned it would be beneficial to determine the occurrence not only of *Clostridium difficile* as well as the more virulent NAP1 strain in order to quantify the burden of disease at our institution. CDI impacts the outcome of ICU patients in a negative manner. By determining whether there is undue delay with regards to diagnosis or initiation of treatment we can improve management of patients. This information can be used to help guide physicians in their decision to treat empirically or await definitive diagnosis.

1.3 Problem statement

Clostridium difficile is not only the leading causal pathogen of antibiotic associated diarrhoea (3) but also the most common causal pathogen for nosocomial infectious diarrhoea (4, 5). ICU patients with CDI carry a higher mortality rate and increased length of ICU and hospital stay compared to patients without CDI (14). Emergence of the more virulent NAP1 strain in the United States, Canada and Europe has increased both incidence and severity of CDI (10). Empiric therapy was shown to be beneficial by shortening the time to resolution of symptoms whilst CDI negative patients gained no benefit (15). South African data regarding CDI are limited. Institutional knowledge regarding CDI occurrence has the potential to improve management of CDI. The positive yield and occurrence of NAP1 strain in stool samples sent for *Clostridium difficile* testing from 576 ICU and 579 HCU at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) was not known. Whether patients with suspected CDI are treated empirically or not was also not known.

1.4 Aims and objectives

1.4.1 Aim

The aim of this study was to determine the positive yield and number of NAP1 strains of all stool samples sent for *Clostridium difficile* testing from 576 ICU and 579 HCU at CMJAH from 1 January 2014 until 31 December 2015.

1.4.2 Objectives

The objectives of this study were to:

- determine the positive yield of *Clostridium difficile* in stool samples sent from 576 ICU and 579 HCU at CMJAH from 1 January 2014 until 31 December 2015
- determine the number of stool samples that were positive for *Clostridium difficile* out of the total number of patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015
- determine the number of NAP1 positive samples
- estimate the time from onset of diarrhoea until the time the stool sample was received in the laboratory
- estimate the time from sample received in the laboratory until laboratory diagnosis
- estimate the time from onset of diarrhoea to initiation of treatment
- determine whether the patient was treated empirically or following laboratory diagnosis
- determine whether a patient was started on metronidazole, vancomycin or both
- determine whether APACHE II score differs between patients with a positive and negative *Clostridium difficile* result

1.5 Research assumptions

The following definitions will be used in this study:

Diarrhoea: The World Health Organization defines diarrhoea as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual) (16).

Stool sample: Faecal matter collected in a sample container for laboratory analysis.

***Clostridium difficile* positive:** Any stool sample that has tested positive either for *Clostridium difficile* toxin A and B using standard enzyme immunoassay or polymerase chain reaction (PCR) or has shown the presence of *Clostridium difficile* on stool culture.

NAP1 strain: *Clostridium difficile* strain that is restriction endonuclease analysis group BI, pulsed-field gel electrophoresis type NAP1 and polymerase chain reaction ribotype 027. It is designated as BI/NAP1/027 (9).

Empiric treatment: Treatment started before a diagnosis is confirmed or refuted.

Clinical records: In this study clinical records will include patient files as well as ICU charts.

Incidence: The number of new cases of a disease, occurring in a specific population during a specific period of time (17).

Prevalence: The total number of cases of a disease at a particular point or period of time in a particular population (17).

1.6 Demarcation of the study field

This study was conducted in 576 ICU as well as 579 HCU at CMJAH. CMJAH is an academic hospital offering a range of secondary, tertiary as well as highly specialised services.

576 ICU is a general medical and surgical adult ICU with 12 beds. The ICU admits approximately 970 patients annually. 579 HCU admits both medical and surgical adult patients. This unit has seven beds and admits approximately 960 patients per year. 579 HCU also serves as a step-down facility from 576 ICU prior to going back to a general ward.

Clinical records included in this study were of all patients admitted to either 576 ICU or 579 HCU for which a stool sample for *Clostridium difficile* testing was submitted to the National Health Laboratory Service (NHLS) from 1 January 2014 until 31 December 2015.

1.7 Ethical considerations

Approval to conduct this study was granted by the Postgraduate Committee (Appendix A) as well as the Human Research Ethics Committee (Medical) of the University of Witwatersrand (Appendix B). Furthermore approval was obtained from the CEO of CMJAH (Appendix C) as well as the Head of Department of Anaesthesiology at CMJAH (Appendix D). Permission to access patient records was obtained from the Director of 576 ICU and 579 HCU at CMJAH (Appendix E). Permission to use the NHLS database was given by the NHLS database gatekeeper (Appendix F).

Data was collected anonymously. Confidentiality of patients was ensured as only the researcher and supervisors has access to the raw data. The data collected will be stored securely for five years. Every patient was a data identification number. The data identification number and hospital number of each patient included in the study was entered on a sheet that was kept separate from the data collection sheet. Only the data identification number was used for data analysis.

This study was retrospective and did not impact clinical management of patients as it did not involve any therapeutic interventions.

This study was conducted in accordance with the Declaration of Helsinki (18) and the South African Good Practice Guidelines (19).

1.8 Research methodology

1.8.1 Study design

A retrospective, descriptive, contextual study design was followed in this study.

1.8.2 Study population

The study population was the clinical records of patients admitted to 576 ICU and 579 HCU for whom stool samples were sent for *Clostridium difficile* test.

1.8.3 Study sample

Sample size

The sample size was determined by the number of available clinical records for the year 2014 and 2015.

Sample Method

Consecutive convenience sampling was used to assess clinical records.

Inclusion and exclusion criteria

Inclusion criteria for this study were:

- The clinical records of all patients over the age of 18 admitted to 576 ICU or 579 HCU who had stool samples sent for laboratory testing for *Clostridium difficile*

Exclusion criteria for this study were:

- Illegible or incomplete records
- In the case were a single patient had more than one sample sent for the same type of test and all results remained the same, only the first sample sent to the laboratory for that particular test was used to estimate the time from onset of diarrhoea until the time the stool sample was received in the laboratory. Similarly only the first sample sent was used to estimate the time from onset of diarrhoea to treatment and whether the patient was treated empirically or following laboratory diagnosis.

1.8.4 Data collection

Data collected

A data collection sheet containing key elements as identified from the literature review was drawn up. The following parameters were recorded:

- Age
- APACHE score
- Clostridium difficile positive/negative
- NAP1 positive/negative
- Number of days in 576 ICU or 579 HCU at onset of diarrhoea
- Time of onset of diarrhoea
- Time of sample received at laboratory
- Time of laboratory diagnosis
- Time treatment initiated
- Empiric treatment yes/no

- Treatment:
  - Oral metronidazole
  - Intravenous metronidazole
  - Oral vancomycin
  - Oral and intravenous metronidazole

Data collection process

All samples sent to the NHLS on an inpatient basis include the patient's ward number on the laboratory requisition form. Following a formal data request, the NHLS provided a list of stool samples as well as results that were sent from 576 ICU and 579 HCU during the period 1 January 2014 until 31 December 2015. Laboratory results were reviewed and data was captured onto the data collection sheet. The relevant patient's ICU chart was obtained and information collected and captured with the aid of the data collection sheet.

1.8.5 Data analysis

Microsoft Excel 2007 was used to create the data collection sheet and capture the relevant data. Descriptive and inferential statistics were used. Categorical variables were described using frequencies and percentages. Statistical analysis was done in consultation with a biostatistician. The Mann U Whitney test was used to determine significance where appropriate, in which case a p-value of <0.05 was considered significant.

1.9 Significance of the study

CDI is the most common causal pathogen for nosocomial infectious diarrhoea as well as for antibiotic associated diarrhoea (3, 4). ICU patients are at higher risk for contracting CDI as well as at increased risk for developing complicated disease. Changes in epidemiology and emergence of the more virulent strains should prompt us to be more vigilant and more aware of *Clostridium difficile* in our institution.

One of the key components of any infection control program is surveillance. Surveillance refers to the routine and consistent collection of data with regards to the occurrence of a disease (20). According to The Study on the Efficacy of Nosocomial Infection Control project (SENIC) surveillance improves the outcomes of patients and is associated with a decrease in nosocomial infection rates (20).

The occurrence of CDI as well as the NAP1 in stool samples sent for suspected CDI in 576 ICU and 579 HCU was not known.

Furthermore empiric therapy has been shown to reduce the time to resolution of symptoms in those who tested positive whilst those that were negative gained no benefit (15). Exploring the treatment patterns at CMJAH 576 ICU and 579 HCU will potentially help to develop management protocols for suspected CDI.

1.10 Validity and reliability of the study

Validity and reliability in this study will be ensured by the following:

- The study design was developed after an extensive literature review
- Data was collected by a single researcher
- Data collection sheets were developed in conjunction with experts in the field namely an intensivist as well as the director of ICU.

1.11 Study outline

The following chapters are presented in this study:

Chapter 1: Overview of the study
Chapter 2: Literature review
Chapter 3: Research methodology
Chapter 4: Results and discussion
Chapter 5: Summary, limitations, recommendations and conclusion

1.12 Summary

In this chapter the background to the study, problem statement, aims and objectives, research assumptions, demarcation of the study field, ethical considerations, research methodology, significance, validity and reliability, and the study outline was discussed. The following chapter, chapter 2, will provide a review of the literature.

Chapter 2: Literature review

2.1 Introduction

This chapter provides a review of the literature with regards to the etiology and epidemiology of *Clostridium difficile,* pathophysiology, NAP1/BI/027 strain, risk factors, clinical features, diagnosis, treatment as well as infection control and prevention.

2.2 Etiology and epidemiology

2.2.1 General

Clostridium difficile is an anaerobic, gram positive bacterium that produces both spores and toxins (1). *Clostridium difficile* was first identified from the stool samples of neonates as early as 1935 and initially named *Bacillus difficilis* due to the difficulty in isolating the organism (5, 21). Only in 1978 did Bartlett et al (2) determine that *Clostridium difficile* was the causal agent of almost all cases of pseudomembranous colitis and 20% of uncomplicated antibiotic associated diarrhoea. Antibiotic associated diarrhoea and colitis has been described since the debut of antibiotics in 1940 (5). *Clostridium difficile* is not only the leading causal pathogen of antibiotic associated diarrhoea (3) but also the most common causal pathogen for nosocomial infectious diarrhoea (4, 5). Whilst most nosocomial infections have declined since 2001, the incidence of CDI has increased (1, 6). This increased incidence can be attributed to the widespread use of broad spectrum antibiotics (3, 7). In several hospitals CDI has surpassed methicillin resistant *Staphylococcus aureus* as the most common cause of nosocomial infection (4). The emergence of the hypervirulent strain of *Clostridium difficile*,NAP1/BI/027 is responsible for the dramatic increase in of *Clostridium difficille* incidence in Europe and North America since 2000 (1, 9, 10) as well as the dramatic increase in mortality and morbidity associated with CDI (5, 10) .

2.2.2 CDI in South Africa

Data regarding CDI as well as the incidence of the NAP1 strain in South Africa is limited, with only a handful of studies identified. The local studies that were identified included a prospective hospital based study (11), a prevalence study that included random samples from both hospital inpatients and primary school children (22) and a study that reported only on laboratory confined disease (11, 22, 23). These studies did not specifically look at intensive care or high care populations.

A prospective study done at Groote Schuur Hospital by Rajabally et al (11) over a period of 15 months found CDI in 9,2 % of patients who either presented to hospital with diarrhoea or developed diarrhoea during their admission. The incidence of nosocomial CDI was 0.087% (11). The incidence of nosocomial CDI in the United States is 0,953% which is notably higher than the incidence found in the aforementioned study (5).

Samie et al (22) reported on polymerase chain reaction (PCR) detection of *Clostridium difficile* in the Vhembe district in South Africa. The study included 322 samples from both hospital inpatients (79.2%) and primary school children (20.8%). Of the 322 samples, 54.6% were diarrhoeal. The total prevalence of *Clostridium difficile* in this study was reported to be 14% (22). Of the diarrhoeal samples, 19.3% were positive for *Clostridium difficile* compared to 7.5% in the non-diarrhoeal samples (22).Toxigenic *Clostridium difficile* was found in 11.4 % of the diarrhoeal samples compared to 2.1% of non-diarrhoeal samples (22).

In 2013 Nana (23) did a prospective study on laboratory confined disease at CMJAH. The study compared two enzyme immunoassay (EIA) and a real-time PCR to toxigenic samples in clinical samples for *Clostridium difficile.* During a one month period, 190 stool samples were included in the study. The number of admissions during the one month study period and the number of toxigenic culture positive samples were used to estimate the prevalence of CDI at CMJAH. There were 1240 admissions during the study period with 42 of the 190 samples toxigenic culture positive resulting in an estimated prevalence of 3.3% (23).

It appears that the incidence of CDI is lower in South Africa than reported in the United States, but there is a need for further investigation in South Africa when one considers the paucity of South African literature.

2.2.3 Diarrhoea in ICU

Diarrhoea in the ICU is a common occurrence and prevalence ranges from 8-38% (12-14) . Patients in ICU are very susceptible to diarrhoea due to the fact that there are multiple factors that reduce their immune response and aggravate diarrhoea (13). There are multiple aetiologies for diarrhoea in ICU and can be broadly classified as infectious, drug induced and non-infectious (12). *Clostridium difficile* is the most common cause of nosocomial infectious diarrhoea (20) and 11% of patients who develop diarrhoea in ICU are diagnosed with CDI (14). Infectious diarrhoea is cause for concern because ICU patients have a greater risk of complications and the causative agent can be spread between ICU staff and patients (4). The intensivist should consider an infectious cause when diarrhoea is associated with fever, severe abdominal pain, vomiting and the presence of blood or mucous in the stool (4). Drug induced diarrhoea is usually secondary to medications such as laxatives, suppositories or enemas (12) as well as non-infectious antibiotic associated diarrhoea. Non-infectious causes of diarrhoea include enteral feeding, low serum albumin levels and intestinal ischaemia (20).

Diarrhoea increases patient morbidity due to malabsorption, fluid losses leading to dehydration and electrolyte disturbances, dermal injury and can have a negative impact on patient dignity (12).The working group on abdominal problems of the European Society of Intensive Care Medicine defined acute gastrointestinal injury as a malfunctioning of the gastrointestinal tract in critically ill patients due to their acute illness (24) . Acute gastrointestinal injury is divided into four grades of severity and diarrhoea is considered grade II gastrointestinal dysfunction (24). Grade II dysfunction is seen when the gastrointestinal tract is unable to digest and absorb adequately to ensure the nutrient and fluid requirements and requires some treatment or medical intervention to meet fluid and nutrient requirements and prevent progression to gastrointestinal failure (24). Gastrointestinal dysfunction is a common occurrence in ICU and is associated with adverse outcome in ICU patients (12).

Diarrhoea increases the burden on nursing staff and increases costs in ICU (12). Diarrhoea is independently associated with increased ICU length of stay and increased mortality (12). On average the length of stay following development of nosocomial diarrhoea increases by eight days (13).

2.2.4 CDI in ICU

Clostridium difficile is the most important cause of infectious nosocomial diarrhoea from an epidemiological view (13). A systematic review conducted by Karanika et al (14) that included 22 studies and 80 835 ICU patients found that 2% of ICU patients contracted CDI whilst in ICU. Incidence of CDI in ICU in the literature ranges from 0.3-3% (12, 14, 25, 26). The prevalence of CDI in the ICU population is significantly higher, nearly double that of the general hospital population (14). Patients in ICU are exposed to multiple risk factors for CDI. ICU patients often receive treatment with multiple and broad-spectrum antibiotics (14). This is due to the fact that infections are the main cause of death in ICU globally (27) and that the risk for developing a nosocomial infection in ICU is 5-10 times greater than in general medical and surgical wards (20). Other risk factors that occur more frequently in ICU than in the general hospital patients arise from the treatment modalities used commonly in the ICU setting such as corticosteroids, gastric acid suppression with proton pump inhibitors and enteral feeding (14). Furthermore critically ill patients often suffer from multiple comorbidities such as renal insufficiency, malnutrition and hypoalbuminaemia that render them immunocompromised and at greater risk for CDI (14).

Patients in ICU have a high risk of adverse events due to complicated CDI (26). The definition of a complicated course of CDI includes death, occurrence of toxic megacolon, need for surgical intervention, admission to ICU and failure to respond to treatment (28). For patients already admitted to ICU septic shock and renal failure requiring renal replacement therapy are regarded as complicated disease (28). CDI also significantly increases not only ICU but also hospital length of stay (14, 29). In the systematic review conducted by Karanika et al (14) regarding length of stay in ICU based on five studies that included 10 327 patients average length of stay for a patient with CDI was 23.54 days compared to 19.16 days for patients without CDI which is statistically significant. This increased length of stay places greater financial strain on health care facilities. This is especially valid in a resource constrained country like South Africa.

CDI in ICU has far reaching implications on both mortality and morbidity. The mortality rate for ICU patients with CDI infection was found to be as high as 32%, which is significantly higher than for those without CDI (14). These figures represent the all-cause mortality associated with CDI and not the attributable mortality of CDI as found by Karanika et al (14) in the systematic review mentioned previously. In this specific systematic review the attributable mortality was found to be approximately 7% (14). Zahar et al (30) found a 6% attributable mortality rate in

their prospective cohort study whilst Kenneally et al (29) found a 6.1% attributable mortality rate. One must take note however that it is very difficult to determine attributable mortality in ICU patients who have multiple comorbidities and that care should be taken to consider confounding variables such as illness severity and adverse events. Comparing mortality rates between different populations and areas are subject to limitations (31). Regional variations in ribotypes, strains and differences in antibiotic resistance has an effect on virulence and may influence the local incidence and mortality rate (31). Risk factors for mortality in ICU include older age, greater severity of illness, lower serum albumin levels, corticosteroids, septic shock and prolonged ICU stay prior to CDI (28, 29). In addition the mortality rate is increased by a primary oncological or medical diagnosis when compared to patients with primary surgical diagnoses (29).

2.3 Pathophysiology

2.3.1 Microbiome and *Clostridium difficile*

In a healthy individual, symbiotes, commensals and pathogens, known collectively as the microbiome, are in equilibrium with the host (27). If the host's physical barriers or immunological defenses are disrupted the equilibrium shifts and this can lead to invasion by colonising bacteria (27).

Prior to the emergence of *Clostridium difficile* as a major enteric pathogen it was thought to be a commensal organism (32). Some studies report that *Clostridium difficile* is a normal gastrointestinal commensal in 1%- 12.9% of healthy individuals (27, 33, 34). In the ICU population, the rate of asymptomatic colonisation with *Clostridium difficile* was reportedly as high as 34.6% (27, 35).

It is not yet proven whether *Clostridium difficile* colonisation has a protective effect or increases the risk of clinical disease (27). Not all individuals who are colonized develop symptomatic disease (32). Most infants are colonized with *Clostridium difficile* yet they do not develop symptomatic colitis (32). One theory suggests that infants lack a toxin-binding receptor in their gut whilst another suggests the development of antibodies to the toxins provides a protective mechanism against symptomatic disease (32). It has been shown that colonization with a toxin

producing strain of *Clostridium difficile* stimulates a lasting immune response that is protective against symptomatic disease later in life (32, 36). The major determinants of developing clinical disease is the virulence of the strain of *Clostridium difficile* and the susceptibility of the host (32).

In healthy individuals colonization is prevented by barrier function and competitive function of normal faecal microbiota (32). Antibiotic exposure is the biggest risk factor for developing CDI and is observed in 96% of individuals that develop CDI during a hospital admission (5). The micobiome is perturbed by antibiotic administration and this promotes the conversion of *Clostridium difficile* spores to a vegetative form that reproduces and produces toxins (5).

Infection is transmitted via spores along the faecal-oral route among humans (32). Spores can exist for years on surfaces (1) and are resistant to alcohol (4).

2.3.2 Virulent strains

Clostridium difficile strains that result in human disease are derived from 39 different ribotypes (37). The 027 strain was the second most common isolate responsible for clinical disease (37). All virulent strains of *Clostridium difficile* carry a 19.6-kb pathogenicity locus (PaLoc) that contains 5 genes: *tcdR, tcdB, tcdE, tcdA* and *tcdC* (9). Toxins A and B are monoglucosyltransferases that are encoded by the *tcdA* and *tcdB* genes (9, 38). Toxin expression is controlled by three regulatory genes (5, 9) which include the positive regulator TcdR and its antagonist TcdC as well as a global regulator, CodY (9, 39).

Pathogenicity is mainly due to toxins A and B as well as the binary toxin (22, 40). The gene encoding for the binary toxin is located outside the PaLoc (22). The binary toxin does show enterotoxicity in vitro (5) but the clinical significance is not yet clear (22).

Toxins A and B enter the host enterocyte where it inactivates the Rho GTPases that play an integral role in structural integrity of the actin cytoskeleton and epithelial barrier function (1, 9). Inactivation of the Rho GTPases leads to collapse of the actin cytoskeleton and apoptosis (1, 9). These changes lead to loss of barrier function and opening of tight junctions (1, 4). Disruption of the tight junctions also allow toxins to come into contact with the submucosa where they elicit upregulation of proinflammatory mediators in the lamina propria and causes a massive

inflammatory response (4). Disruption of tight junctions result in increased permeability and fluid accumulation in the intestinal lumen and ultimately manifests clinically as diarrhoea (1, 4).

2.4 NAP1/BI/027 Strain

2.4.1 General

In recent years *Clostridium difficile* has gained some notoriety following the emergence of the North American pulsed field gel electrophoresis type 1 (NAP1) strain (8). NAP1/BI/027 is responsible for the dramatic increase in of *Clostridium difficille* incidence in Europe and North America since 2000 (1, 9, 10) as well as the dramatic increase in mortality and morbidity associated with CDI (5, 10) . This strain is more likely to be refractory to treatment and has an increased frequency of relapse (5)

2.4.2 Hypervirulence

The strain is classified as restriction endonuclease analysis group BI, pulsed-field gel electrophoresis type NAP1 and polymerase chain reaction ribotype 027 and carries the designation NAP1/BI/027 (9).

Hypervirulance of this strain is due to multiple factors such as increased toxin production and high level resistance to fluoroquinolones (5). NAP1/BI/027 produces 16 times more toxin A and 23 times more toxin B in vitro compared to historical control (5, 11, 41). Increased production of toxins is attributed to a deletion of a base pair at position 117 of the *tcdC* gene which acts as a repressor of toxin production (5, 9).

NAP1/BI027 not only produces toxin A and B but also a third toxin called *Clostridium difficile* binary toxin (CDBT) (9). It is composed of two subunits encoded by the genes *cdtA* and *cdTB* that encodes for the enzymatic and binding component respectively (9). The genes coding for CDBT are located outside the PaLoc region in a region called CdtLoc (9). Although the clinical significance of CDBT is not yet clear it has been proposed that it has a synergistic effect with toxins A and B and increases their virulence (9, 42).

Due to the fact that historically NAP1/BI/027 was sensitive to fluoroquinolones, it has been widely assumed that acquisition of resistance to these agents has played a major role in its emergence as an epidemic strain (5).

During the outbreak in Quebec in 2004 the incidence and mortality rate showed a nearly four-fold increase when compared to 1997 (9). In some hospitals in Quebec development of complicated severe CDI increased 10-fold (9). Between October 2003 and June 2005 there were two outbreaks of CDI in southeast England with an attributable mortality of 11% (9). More than 59% of the isolates responsible for that outbreak were 027 strains (9). The 027 strains were first observed in Hardewijk in the Netherlands in 2005 when they experienced a 21-fold increase in CDI with a 6% attributable mortality (9).

2.4.3 NAP1/BI/027 in South Africa

A prospective study done at Groote Schuur Hospital by Rajabally et al (11) over a period of 15 months found that out of the 643 patients included in this study only two cases of the NAP1 strain were reported.

Samie et al (12) reported on PCR detection of Clostridium difficile in Vhembe district in South Africa. The study included 322 stool samples from hospital in patients as well as out-patients and reported the molecular characterization of *Clostridium difficile* in their district (22). Globally toxin A was found in 40% , toxin B in 46.7% binary toxin genes were found in 26.7% of samples (22). The binary toxin in isolation was only found in three of the samples (22). It appears that the incidence of the NAP1/BI/027 strain is very rare in South Africa but there is a need for further investigation in when one considers the paucity of South African literature.

In 2013 Nana (23) did a prospective study on laboratory confined CDI at CMJAH. 190 samples were included in the study and 43 were found to be toxigenic culture positive (23). Not one of the 43 toxigenic culture positive were found to have the tcdC gene deletion associated with the 027 ribotype (23).

2.5 Risk factors

2.5.1 General risk factors

The major determinants of developing clinical disease are the virulence of the strain of *Clostridium difficile* as discussed previously and the susceptibility of the host (14).

General risk factors associated with developing CDI can be broadly grouped into three categories: disruption of normal intestinal flora and gastrointestinal immune function, environmental contamination and host factors (4)(Table 2.1).

Table 2.1 General risk factors for CDI

Disruption of intestinal flora/mucosa or immune suppression	**Environmental contamination**	**Host factors**
Antibiotic treatment	Length of stay in healthcare facility	Age > 65 years
Fluoroquinolone-resistant BI/NAP/027 strain	Possible food contamination	Multiple comorbidities
Proton pump inhibitors and histamine 2 (H2) receptor antagonists		Peripartum women and children
Chemotherapy		Inflammatory bowel disease
Glucocorticoids		HIV
Radiation		Chronic kidney disease requiring haemodialysis
Intestinal stasis		Low serum albumin levels
Abdominal surgery		
Nasogastric tubes and Enemas		

(4)

2.5.2 Major risk factors

The major risk factors for developing CDI are antibiotic exposure, advanced age and hospitalisation (5, 43).

Antibiotic exposure

Antibiotic exposure is the most significant modifiable risk factor for developing CDI (1, 4, 5). Perturbation of normal competitive flora in the gastrointestinal system results in overgrowth of *Clostridium difficile* and production of toxins (4). Almost all antibiotic classes have been implicated but cephalosporins, clindamycin and fluoroquinolones are frequently associated with CDI (44, 45). Penicillins, macrolides, sulphonamides and trimethoprim are associated to a lesser degree (46, 47).

Paradoxically numerous antibiotics that are causal agents for CDI demonstrate activity against *Clostridium difficile* in vitro (32). Even metronidazole and vancomycin, the agents most commonly used for the treatment of CDI, have been implicated as causal agents for CDI (15). Antifungals and antivirals have been infrequently associated with the development of CDI (15).

Age

Age is a major risk factor for CDI (4). The risk of CDI, severity of illness, frequency of complications and recurrences increase as age increases (5, 32). In various studies, the risk of developing CDI was up to 10 times higher in patients above the age of 65 when compared to younger patients (5, 32). The higher risk in the older patient might be attributed to increased number of comorbidities in association with increased use of antibiotics or might be due to the deterioration of the immune system (4).

Length of stay

Risk of developing CDI correlates directly with length of hospital stay (5). Patients admitted to hospital between one and three weeks have a 15 - 45% risk of becoming colonized with *Clostridium difficile* (5). Patients are infected mainly by spores present on the hands of health care professionals (4). Increased length of stay not only increases exposure to spores but also increases the risk of antibiotic exposure (4).

2.5.3 Gastric acid suppressants

Recently the use of gastric acid suppressants such as proton pump inhibitors and H2-receptor antagonists have become a matter of interest as a risk factor for developing CDI. At this time

results are conflicting. Some studies report no increased risk with gastric acid suppression, increased risk with proton pump inhibitors only but associated with a dose response, or a higher risk when proton pump inhibitors and H2-receptor antagonists are used in combination (4).

The mechanism for increased resistance of *Clostridium difficile* to gastric acid suppression is not yet known (4). Vegetative forms of *Clostridium difficile* survive longer in gastric contents where the pH has been increased to five by addition of acid suppression but these vegetative forms do not survive in ambient air for more than 15 minutes and are not likely to be ingested (4). However stool of patients with CDI contain ten times as many vegetative forms compared to spores and might explain the association of proton pump inhibitors and H2-receptor antagonists with CDI (48). The spores that act as the vectors for infection are much more likely to be ingested as they are able to survive on surfaces for years (1). The spores that are ingested are acid stable and remain viable at normal gastric pH (32). Some theories regarding the increased CDI risk are that increased gastric pH disrupts the normal flora of the upper gastrointestinal tract and as a result the microbiome in the lower gastrointestinal tract is altered that leads to overgrowth of *Clostridium difficile* (4). Irrespective of the pathophysiological mechanism, nearly 50% of patients on gastric acid suppression have no indication for it and discontinuation of it should be considered in the presence of primary or recurrent CDI(4).

2.5.4 APACHE II

The Acute Physiology And Chronic Health Evaluation Scoring System (APACHE) was developed in 1981 at the George Washington University Medical Centre in an attempt to quantify disease severity (49). A reliable severity classification system that accurately predicts outcome can be used to optimize ICU bed usage by excluding low risk patients as well as futile care for the terminally ill (50). It was found that the APACHE score correlated directly with mortality (50). In 1985 the APACHE II score was introduced as a simplified version of the original (51).

The APACHE II system consists of three parts: 12 acute physiological variables, age and chronic health status as seen in table 2.2 (50). The most abnormal values during the first 24 hours in ICU are used for the acute physiology score (50). Age points scored from zero to six (50).

Table 2.2 The APACHE II Scoring System

Physiological variable	High abnormal range					Low abnormal range				
	+4	+3	+2	+1	0	+1	+2	+3	+4	Points
Temperature	≥41°	39 to 40.9°		38.5 to 38.9°	36 to 38.4°	34 to 35.9°	32 to 33.9°	30 to 31.9°	≤ 29.9°	
Mean Arterial Pressure – mmHg	≥160	130 to 159	110 to 129		70 to 109		50 to 69		≤49	
Heart rate	≥180	140 to 179	110 to 139		70 to 109		55 to 69	40 to 54	≤39	
Respiratory rate	≥50	35 to 49		25 to 34	12 to 24	10 to 11	6 to 9		≤5	
Oxygenation a.FIO2 ≥ 0.5 record A-aDO2	≥500	350 to 499	200 to 349		<200					
b. FIO2 <0.5 record PaO2 (mmHg)					PO2> 70	PO2 61 to 70		PO2 55 to 60	PO2<55	
Arterial pH	≥7.7	7.6 to 7.69		7.5 to 7.59	7.33 to 7.49		7.25 to 7.32	7.15 to 7.24	<7.15	
Serum HCO3 (venous mEq/l)	≥52	41 to 51.9		32 to 40.9	22 to 31.9		18 to 21.9	15 to 17.9	<15	
Serum sodium (mEq/l)	≥180	160 to 179	155 to 159	150 to 154	130 to 149		120 to 129	111 to 119	≤110	
Serum potassium (mEq/l)	≥7	6 to 6.9		5.5 to 5.9	3.5 to 5.4	3 to 3.4	2.5 to 2.9		<2.5	

Serum creatinine (mg/dl)	≥3.5	2 to 3.4	1.5 to 1.9		0.6 to 1.4		<0.6			
Haematocrit (%)	≥60		50 to 59.9	46 to 49.9	30 to 45.9		20 to 29.9		<20	
White Blood Count (total/mm³)	≥40		20 to 39.9	15 to 19.9	3 to 14.9		1 to 2.9		<1	
Glasgow Coma Scale (GCS) Score =15minus actual GCS										
A. Total Acute Physiology Score (sum of the above 12 points) **B. Age points (years)** ≤44=0, 45 to 54=2, 55 to 64=3, 65-74-=5, ≥75=6 **C. Chronic health points** (see below) Total APACHE II Score : add together points from A+B+C										

(51)

Chronic health points are weighted zero to five and are given on the basis of a history of severe organ insufficiency or immune compromise (50). Nonoperative or emergency postoperative patients are assigned five points and elective postoperative patients are assigned two points if severe organ insufficiency or immune compromise is present (50). Severe organ insufficiency is divided in to organ systems as seen in table 2.3.

Table 2.3 Organ insufficiency

Organ system	Organ insufficiency
Liver	Biopsy proven liver cirrhosis and documented portal hypertension
Cardiac	New York Heart Association Class IV cardiac failure
Respiratory	Chronic obstructive, restrictive or vascular disease resulting in severe exercise restriction or documented chronic hypoxia, hypercapnia, secondary polycythemia or severe pulmonary hypertension
Renal	Patients receiving chronic dialysis

(50)

Immune compromise includes patients receiving chronic immune suppression, chemotherapy, radiation, long term or recent high dose steroids and patients with AIDS, leukemia and lymphoma (50). The uses of the APACHE II score include risk stratification, comparing quality of care in ICU and prognostication (50).

ICU patients are at increased risk for contracting CDI (14). In ICU, higher APACHE II scores are considered a risk factor for developing CDI (52). In general higher APACHE II scores reliably predict a higher mortality in critically ill patients(14). Higher APACHE II scores have also been shown to be predictive of developing CDI (14, 29). The APACHE II scores of patients with CDI in our ICU are not known.

2.6 Clinical features

2.6.1 General

Similar to other gastrointestinal pathogens CDI can present with a wide spectrum of clinical manifestations (1). Clinical presentation ranges from asymptomatic colonization, mild or moderate diarrhoea up to fulminant colitis and death (1, 53)**.**

2.6.2 Severity of disease

CDI can be divided into mild, moderate and severe disease based on clinical and laboratory findings. (Table 2.4)

Table 2.4 Severity of disease

	Mild disease	**Moderate disease**	**Severe disease**
Diarrhoea	Mild, bloodless (32, 53)	Bloodless (53)	Severe or bloody
Colitis	No	Yes (53)	Yes (32, 53)
Other symptoms		Dehydration (32)	Vomiting Ileus Fever > 38.9ºC (32, 53)
Laboratory abnormalities	None	Urea and creatinine above baseline (53)	White cell count> 15 000/µl Albumin <3g/l Creatinine > 1.5 premorbid level or acute kidney injury (1, 53)

Symptoms of colitis include fever, abdominal pain and cramping or urea and creatinine raised above baseline (53).

2.6.3 Fulminant colitis

Fulminant colitis is a feared complication of CDI and occurs in 2% to 5% of patients (1, 4). Fulminant colitis is frequently referred to as complicated CDI (CCDI). CCDI is characterized by severe abdominal pain and distention, worsening of diarrhoea and the presence of one or more of the following: haemodynamic instability, toxic megacolon, intestinal perforation, peritonitis, the need for ICU admission, altered mental status, end organ failure (usually respiratory or renal failure), temperature >38.5ºC, white cell count >35 000/µl or serum lactate >2.2mg/dl (1, 5, 32).

Pseudomembranous colitis and toxic megacolon are the hallmark of CCDI (4). Although diarrhoea is usually required to diagnose symptomatic CDI, the lack thereof in a patient with worsening abdominal distention and clinical deterioration suggests ileus associated with toxic megacolon (1, 4).

CCDI seems to occur more frequently in patients with malignancies, renal failure, immunosuppression or patients receiving antiperistaltic agents but the most consistent predictors of CCDI have been age, a raised white cell count and increasing creatinine levels (5).

Complications of fulminant colitis include multi organ failure, abdominal compartment syndrome and adult respiratory distress syndrome and ultimately death (4). About 50% of patients admitted to ICU with CCDI develop septic shock associated with multi organ failure (5). Several animal studies have suggested that complications of fulminant colitis could be due to toxaemia and abnormal modulation of the immune response (4). Bacteraemia due to translocation of gastrointestinal bacteria occurs in only 4% of patients (5). Hence the systemic inflammatory response seen in CCDI is not due to bacteraemia but rather from *Clostridium difficile* toxin-induced inflammatory mediators such as interleukin-8, macrophage inflammatory protein 2, substance P and tumour necrosis factor α that are released locally in the gut (5, 54, 55).

Fulminant disease carries a mortality rate of between 30% to 90% (1, 56). The high mortality rate associated with CCDI is usually due to delay in the diagnosis of fulminant colitis (4). The lack of a reliable prediction model to identify patients at increased risk for the development of CCDI remains a clinical problem (57). Currently a number of inflammatory markers are the topic of interest as possible predictors of CCDI or death. Faecal interleukin-8 and its stimulator mitogen-activated protein kinase 2 have been associated with CDI (4).

2.7 Diagnosis

2.7.1 Laboratory diagnosis

Diagnosis of CDI is made by identifying the presence of the *Clostridium difficile* toxin in stool (5). Usually the presence of the organism in stool is of little value due to the high rate of asymptomatic colonisation in the hospital population (5). Between 40-60% of hospitalized patients are colonised with *Clostridium difficile* in the absence of clinical disease (4).

Asymptomatic carriers of *Clostridium difficile* will yield false positive results and as such laboratory testing should be reserved only for patients who present with symptoms of CDI and only on diarrheal stool.

The ideal diagnostic test should have a high sensitivity and specificity, quick turnaround time and be affordable. There are numerous methods for diagnosing *Clostridium difficile* in stool but each has their limitations. Numerous laboratory tests can confirm the presence of a toxin producing *Clostridium difficile* but significant discrepancies have been found and currently there are no existing diagnostic guidelines. Toxigenic culture is considered the gold standard to identify toxin producing strains (1, 5, 53). Diagnostic tests include enzyme immunoassays (EIA) for toxins as well as the *Clostridium difficile* common antigen glutamate dehydrogenase (GDH), nucleic acid amplification tests (NAAT) such as polymerase chain reaction for *Clostridium difficile* toxin genes, DNA microarray and loop-mediated isothermal amplification and cell culture neutralization assays (CCNA) (1, 53).

Cell neutralization culture assay

Toxins A and B are important virulence factors and the majority of tests aim to confirm their presence in stool (58). Cytotoxin neutralisation or cell culture neutralization assay was developed in the 1970's and was the first test to detect toxin B on the culture medium after incubation (58). Stool samples are suspended in a medium which is then filtered to separate the toxins from the organism and viruses (1). The filtrate is added to tissue (human fibroblasts) either with or without antibodies to toxin B, incubated and then monitored for neutralisation or cytopathic changes (1, 5). Specimens that are negative for *Clostridium difficile* toxin B will remain unchanged in both samples that contain antibodies to toxin B and those that do not (1). In samples without antibodies, the specimen that is positive for toxin B will cause cytopathic changes to the fibroblasts (1, 5). In those samples with antibodies the toxin will be neutralised and no cytotoxic changes will be present (1). This test has a very high specificity, almost 100%, but sensitivity ranges from 75-100% (1). Cytotoxin neutralisation assay is time consuming and not widely available (58).

Toxigenic culture

Toxigenic culture involves culture of the organism on an selective aerobic medium for two to five days (58). After *Clostridium difficile* is identified, capacity for toxin production is demonstrated by testing for toxin producing genes such as *tcdB* that produces toxin B (1, 5). Toxigenic culture

has a high sensitivity (94-99%) and high specificity (99%) (1) and for that reason remains the gold standard in diagnosing toxigenic strains of *Clostridium difficile* (1, 53, 58). There is concern regarding false positives in asymptomatic carriers (58). The problem with toxigenic culture is that it has a long turnaround time of two to five days, it is labour intensive, requires skilled laboratory expertise and is not widely available (1, 58).

Enzyme immunoassay: toxin A and B

During the 1990's enzyme immunoassays was developed for the detection of *Clostridium difficile* toxins A and B (58). It became the standard diagnostic test for CDI until the early 2000's (58) and is still in use. Some *Clostridium difficile* strains are toxin A negative and assays detecting both toxins are favored (5). Stool samples are added to microwells coated toxin A and B polyclonal antibodies (1). In the presence of the *Clostridium difficile* toxins complexes of antibodies and toxins form and are identified by a calorimetric reaction (1).

EIA has a turnaround time of two to six hours which is much faster than a cell neutralisation culture assay but has a much lower sensitivity (1, 5). Reported sensitivity ranges between 45% and 93% (5, 58) with a specificity of between 75% and 100% (5). At present EIA for toxins is not considered sensitive enough to be used as a stand-alone diagnostic test (58). Currently EIA for toxin A and B is used in conjunction with the EIA for GDH as a initial screening test (5).

Enzyme immunoassay: glutamate dehydrogenase

GDH EIA came onto the market in 2006 and was initially used as a diagnostic test (58). Glutamate dehydrogenase is a common cell wall antigen of *Clostridium difficile* and is secreted in stool (1, 58). In microorganisms GDH converts glutamate to α-ketoglutarate (1). GDH is nonspecific and does not confirm the presence of a *Clostridium difficile* strain but in the absence of GDH *Clostridium difficile* is highly unlikely (1). It has a negative predictive value of 99% to 100% (1,58). The sensitivity of this test is 87% to 90% and the positive predictive value is 63% (1). The high negative predictive value is the main reason why it is not used as a diagnostic test and is mostly used for screening. All samples that are positive for CDI will then be investigated further with a second test with a higher sensitivity.

When CDI is suspected a sample will be sent for GDH as a screening test due to its high negative predictive value. If GDH is positive an EIA for toxins A and B will be done. This is referred to as the multistep approach (58). The multistep approach has demonstrated a high

negative predictive value as well as sensitivity (5). GDH and EIA has been combined and made commercially available as a single confirmatory test called the C.DIFFQUIK CHEK Complete assay manufactured by Techlab in Blacksburg, Virginia and has a turnaround time of about 30 minutes (58). Combining the two tests is considered sufficient to diagnose CDI (58).

Nucleic acid amplification tests

Further progress was made in diagnosing CDI in 2009 when the Nucleic Acid Amplification Test (NAAT) for CDI was released onto the market (58). NAAT detects the genes coding for toxin B production, the *tcdB* gene, following DNA amplification (1, 58). NAAT includes six PCR-based assays as well as DNA microarray and loop-mediated isothermal amplification (1, 58). These tests have a fast turnaround time of between 45 minutes and two hours and reports either a positive or negative result (1). Sensitivities of these assays range from 77% to 100% and have specificities between 87% and 100% (1, 58).

The major benefit of these tests is the rapid diagnosis. When GDH and toxin EIA as a multistep approach is compared to NAAT, results are obtained faster as NAAT is a real-time test (58). There are some data to support that faster diagnosis can lead to reduction in CDI complications such as admission to ICU, colectomy and death (58). Furthermore earlier diagnosis results in a reduction in duration of empiric antibiotic therapy and isolation precautions (59).

The disadvantage of the NAAT is that the test only detects the gene coding for toxin B and not the presence of the toxin itself. Thus the test can be oversensitive and false positives can occur (58). The worry is that currently CDI might be over-diagnosed and many patients are asymptomatic carriers are being treated with antibiotics (58).

Laboratory diagnosis at CMJAH

Currently the CMJAH microbiology laboratory offers both toxin A and B immunoassay as well as a real-time PCR assay that was introduced in the Infection Control Services Laboratory (23). The PCR being used at the Infection Control Services Laboratory at CMJAH is the Xpert *C.difficile* Assay (GeneXpert, Cepheid, Sunnyvale, CA, USA) performed on the Cepheid GeneXpert ® System. The actual test process takes 45 minutes and does not require batch testing (60). The test has a sensitivity of 93.5% and a specificity of 94.0% (60).

The immunoassay used at CMJAH is the Meridian Immuno Card™ Toxins A & B assay (Meridian Bioscience, Cincinnati, OH). This assay is simple to perform, batch testing is not required and the actual test process takes 35 minutes (23).

A prospective study by Nana (23) from the Department of Microbiology at CMJAH in 2013 on 190 stool samples sent for Clostridium difficile test found the sensitivity of the EIA to be only 38% but the specificity was 100%. Unfortunately, at that time the aforementioned study was conducted, the GeneXpert® System was not yet available at CMJAH to compare test performance.

2.7.2 Imaging studies

Abdominal computed tomography

Radiological investigations for CDI are neither sensitive nor specific (53). Abdominal computed tomography (CT) signs suggestive of severe colitis are diffuse colonic thickening, the target sign (multilayered appearance of edematous mucosa), the accordion sign (thickened haustra seen as alternating bands of high and low density) and pericolic stranding (1). The accordion sign can also suggest CDI (1). Colonic thickening in patients with CDI more often involves the descending colon (5).

Advantages of CT is that is noninvasive, results are available immediately and other abdominal pathology can be excluded and it can be used to track disease course (1). The major disadvantage of CT is that it is neither specific nor sensitive for CDI (53).

Endoscopy

Endoscopy can be used when laboratory results are negative or inconclusive but the clinical presentation of the patient is in keeping with CDI (53). It can be a useful aid when the differential diagnosis includes other colonic causes (53).

The pathognomonic feature of CDI is the presence of pseudomembranes on the colonic mucosa (1). Pseudomembranes are raised, two to 10 mm white or yellow plaques occurring on erythematous, edematous colonic mucosa (1). The plaques consist of inflammatory cells, fibrin and other cellular debris (1).

Although pseudomembranes are pathognomonic of CDI, they are only seen in 50% to 60% of patients and as a result, endoscopy can have false negative rates up to 25% (1). In light of low sensitivity, high costs, patient discomfort and procedural risks such as colonic perforation, endoscopy is not a popular diagnostic tool for CDI (1).

2.8 Treatment

2.8.1 General principles

The strongest recommendation in the initial management of CDI is to stop all potential causal antibiotics or medications (53, 58). During the 1980's it was reported that 15% to 23% of patients' symptoms resolved spontaneously by withdrawing the causal antibiotic without initiating specific therapeutic antibiotics (61). This approach is not adopted in clinical practice due to the difficulty in predicting which patients will develop complicated disease. It might be impossible to stop all antibiotics in the face of concurrent infection but in such cases an attempt should be made to avoid cephalosporins, clindamycin and fluoroquinolones that are frequently associated with CDI (1, 46, 47). It might be difficult to determine the causal agent in ICU patients due to polypharmacy practiced in ICU.

Supportive management includes correction of electrolyte derangements and hydration to maintain euvolaemia as patients with moderate and severe disease can become dehydrated and haemodynamically unstable.

Anti-motility agents in the setting of CDI are not recommended (53). Toxins produced by *Clostridium difficile* disrupt the intestinal mucosa and result in inflammatory infiltration of the lamina propria (4, 62). Anti-motility agents increase the contact time between toxins and the intestinal barrier epithelium by decreasing peristalsis (62). It is believed that anti-motility agents may increase the risk for complicated disease and mortality (62).

Gastric acid suppression in the context of CDI whether by proton pump inhibition or H2-receptor antagonism is a contentious matter and may pose additional risk. Gastric acid suppressants should be reviewed and only continued if there is a clear indication.

Strict infection control precautions including isolation, barrier protection and hand washing should be initiated as soon as the diagnosis is made.

2.8.2 Medical management: Antibiotics

Metronidazole and vancomycin: First episode

Medical management of CDI is based on severity of clinical disease and whether it is the first presentation or a recurrence.

Clostridium difficile remains within the colonic lumen and successful treatment relies on delivery of an antibiotic with antimicrobial activity against the organism to this site (5). Metronidazole and oral vancomycin are the main antimicrobials used for the treatment of CDI since the 1970's and despite their frequent use no clinically significant resistance has developed to either of these antimicrobials (32).

Vancomycin is the only antimicrobial approved for the treatment of CDI by the United States Food and Drug Administration (FDA) (5, 63). Oral vancomycin has ideal pharmacological properties for the treatment of CDI. It is poorly absorbed and remains within the intestinal lumen where it can reach levels of up to 100 times the minimum concentration required to inhibit bacterial growth (5). Due to its poor absorption following oral administration there is a relatively small risk of systemic toxicity (64). There are several concerns regarding the use of vancomycin as first line therapy for all cases of CDI. Firstly, there are concerns regarding the emergence of vancomycin-resistant enterococci with frequent, indiscriminate use of vancomycin in the hospital setting (5, 63). Secondly, the oral formulation of vancomycin is much more expensive compared to the generic intravenous formulation and metronidazole (64).

Oral metronidazole has a very rapid and nearly complete absorption in healthy adults resulting in undetectable levels in stool (61, 64). Oral metronidazole has been found to be effective in mild to moderate disease resulting in clinical resolution of disease (61). In the case of symptomatic disease levels of metronidazole in the stool are significantly higher than compared to levels in health (61). Higher levels of metronidazole in watery or diarrhoeal stool might indicate an increased gastrointestinal transit time that results in decreased absorption or leakage of drug containing plasma across the more permeable and inflamed mucosa (61).

Parenteral metronidazole is found in higher concentrations in diarrhoeal or watery stool that occurs during symptomatic disease when compared to formed stool (61). Thus intravenous metronidazole has an important place in treatment of CDI in the ICU and in postoperative patients (61).

Two older randomised control trails did not show any difference in clinical cure or recurrence rates when comparing vancomycin and metronidazole. (65-67). Neither of these studies were blinded nor were patients stratified according to severity of disease. The first prospective, randomized, double-blind, placebo-controlled trail by Zar et al (68) comparing vancomycin with metronidazole for the treatment of CDI, showed that vancomycin was generally superior to metronidazole but treatment benefit was only seen in patients with severe disease. In the case of severe infection, vancomycin demonstrated a cure rate of 97% versus 76% of patients treated with metronidazole (32, 68). In patients with mild disease, differences in cure rates were not statistically significant (32, 68). Some studies have found higher rates of clinical failure with metronidazole following the outbreak of the NAP1/BI/027 strain (69). Thus for mild or moderate disease metronidazole and vancomycin are considered to be equivalent (32, 68). The main concern when deciding on treatment based on severity is that there is no validated means of predicting which patients with mild disease will progress to severe disease.

Currently, both the American College of Gastroenterology (ACG) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommend the use of oral metronidazole (500mg) three times daily for 10 days for the treatment of the first episode of mild to moderate CDI (65, 70) (Table 2.3). In the presence of severe disease without significant abdominal distention, both ACG and ESCMID recommend the use of oral vancomycin (125mg) four times daily for 10 days (65, 70). For the treatment of severe disease associated with significant ileus or abdominal distention, the ACG recommends the use of vancomycin (500mg) orally and vancomycin (500mg) diluted to 500ml per rectum four times daily (65). In addition, they recommend the addition of metronidazole (500mg) given four times daily intravenously (65). The ESCMID recommends vancomycin (125mg) four times daily for 10 days in this patient group (70). No significant difference was found in cure rates when high dose vancomycin (2 g in four divided doses per day) was compared with low dose vancomycin (500mg in four divided doses per day) (71). Both ACG and ESCMID recommend surgical consultation in severe disease (65, 70).

The main concern is that drugs will not reach the colonic lumen if there is impaired gastric motility. Higher doses and volumes of vancomycin are used than previous recommendations based on the theory that higher volumes increase the probability that the antibiotic will reach the proximal colon (65). Direct instillation of vancomycin into the colon either by enema, colonoscopy or long rectal tube has been effective in small series and case reports (72, 73).

There are no randomised controlled trials available with recommendations for choice and dosage of antibiotics specifically for severe disease and most recommendations are extrapolated from clinical experience and data from recurrent CDI (65).

Table 2.5 Recommendations from the ACG and ESCMID for the management of the first episode of CDI

CDI severity	ACG guidelines (2013)(65)	ESCMID guidelines (2014)(70)
Mild-moderate	Metronidazole 500mg t.d.s PO for 10 days If unable to tolerate metronidazole or no improvement after 5-7 days: Vancomycin 125mg q.i.d PO	Metronidazole 500mg t.d.s PO for 10 days Alternatives Vancomycin 125mg q.i.d PO for 10 days Fidaxomicin 200mg b.d PO
Severe	Vancomycin 125mg q.i.d PO	Vancomycin 125mg q.i.d PO Alternative Fidaxomicin 200mg b.d PO
Severe and complicated (abdominal distension or ileus)	Vancomycin 500mg q.i.d PO plus metronidazole 500mg t.d.s IV Vancomycin 500mg in 500mls q.i.d per rectum Surgical consult/ management	Vancomycin 125mg q.i.d PO Alternative Fidaxomicin 200mg b.d PO Surgical consult/management

Fidaxomicin

In 2011 fidaxomicin was approved by the FDA) for the treatment of CDI (32). Fidaxomicin is a macrocyclic antibiotic with bactericidal activity against specific anaerobic gram positive bacteria

(32). In the case of *Clostridium difficile,* it inhibits clostridial ribonucleic acid (RNA) polymerase (45). Like vancomycin, fidaxomicin is minimally absorbed when taken orally. In contrast to metronidazole and vancomycin it does not disrupt the gastrointestinal microbiome (45). Normal commensals such as the *Bacteroides* species, which may be protective against *Clostridium difficile,* are resistant to fidaxomicin (4).

In clinical trials fidaxomicin was found to have equivalent cure rates compared to vancomycin in mild or moderate disease (74, 75). There is, however, no data regarding the efficacy of fidaxomicin in severe or complicated disease (65). Recurrence risk for patients treated with fidaxomicin was found to be significantly lower than those patients with mild or moderate disease treated with vancomycin (74, 75). Fidaxomicin did not reduce the risk of recurrence in patients infected with the NAP1/BI/027 strain (32).

At this point in time fidaxomicin is not first line therapy for CDI largely due to its markedly higher cost (1).

Other antibiotics

Rifaximin

Rifaximin is a synthetic antibiotic that has gram-positive and gram-negative effect in vitro and is poorly absorbed following oral administration (61). It has been proven as an effective treatment for traveler's diarrhoea (61). Studies using rifaximin for the treatment of CDI are limited. Rifaximin has been used primarily in the treatment of recurrent *Clostridium difficile* infection RCDI as a two week chaser course following vancomycin. A small retrospective study found that rifaximin has a reasonable effect in the treatment of CDI and can be used as an optional treatment for RCDI (76). A randomised, double-blind, placebo-controlled pilot study by Garey et al showed that patients who were treated with a two week chaser regimen of rifaximin following vancomycin had a decreased incidence of RCDI when compared to placebo (77). There are growing concerns regarding resistance to rifaximin especially in patients who has previously received the drug (45). Currently rifaximin is not recommended as first line therapy due to a lack of data and concerns regarding antimicrobial resistance. Rifaximin can be considered only as an optional treatment for RCDI.

Nitazoxanide

Nitazoxanide is used to treat helminthic and protozoan infections and is effective against *Clostridium difficile* in vitro (61). Approximately 66% of the drug is excreted in the faeces as tizoxanide, an active metabolite with the same antimicrobial activity as nitazoxanide (61). A small prospective randomized control trial comparing vancomycin with nitazoxanide showed at least similar efficacy but unfortunately the patient numbers in the trail were too small to comment on noninferiority (78). Further studies need to be done before treatment recommendations can be made.

OPT-80 (difimicin)

OPT-80 is another antimicrobial with poor intestinal absorption, activity against *Clostridium difficile*, very little effect on other gram-positive anaerobes and no effect on gram-negative organisms (5, 61). OPT-80 has ideal characteristics for the treatment of CDI and is the most promising emerging drug. If it proves to be superior to vancomycin in clinical trials it would be a significant advancement in the treatment of CDI.

2.8.3 Medical management: Agents that bind the toxin

Colestipol and cholestyramine

Colestipol and cholestyramine are anion exchange resins that bind the toxins produced by *Clostridium difficile* in vitro but seem to lack any clinical effect (5, 61). Furthermore these drugs bind to other drugs used to treat CDI such as vancomycin making it obsolete for clinical practice (5, 61).

Tolevamer

Tolevamer is a nonantibiotic anionic polymer that binds toxins A and B in vitro without interfering with the absorption of other drugs (5, 61). Results of phase three trials in a double-blind, multicentre study showed that tolevamer was clearly inferior to both vancomycin and metronidazole (5, 79). It did however show that if a patient had resolution of disease with tolevamer that recurrence was uncommon (79).

2.8.4 Medical management: Antibody to the toxin

Higher levels of anti-toxin antibodies are associated with shorter disease duration and lower recurrence rates (53). A randomised, double-blind, placebo-controlled trial comparing the recurrence rates of two monoclonal antibodies against *Clostridium difficile* toxin A and B to placebo found a significantly lower rate of recurrence in patients treated with the monoclonal antibodies in addition to metronidazole or vancomycin (80). Antibodies to toxin A are thought to offer the most protection against recurrence (53, 81).

2.8.5 Recurrent disease

Recurrence is defined as total resolution of initial symptoms after appropriate treatment with subsequent return of symptoms within eight weeks after initial treatment has been completed (53). Following an initial episode of CDI between10% to 20% of patients relapse (53, 65, 82). Once a patient has a recurrence the risk of further recurrences increase to 40% to 65% (53, 65, 82). Recurrence can be due to relapse by the same strain or re-infection with a different strain (83).

Recurrences are attributed to impaired immune responses or disruption of normal gastrointestinal flora (65). In patients with RCDI there is a significant decrease in the diversity of the faecal microbiome (58).

The ACG recommends that the first recurrence of CDI should be treated with the initial standard regimen unless severe, in which case vancomycin should be used (65). Recurrence rates following treatment with metronidazole and vancomycin are similar but recurrences following the use of fidaxomicin are less (70). The evidence for fidaxomicin is limited to retrospective studies with limited patient numbers (70). Therefore the ESCMID recommends using the standard vancomycin, metronidazole or fidaxomicin regimens for the first recurrence (70).

For second recurrences both the ACG and ESCMID recommends a pulsed or tapered regimen with vancomycin (Table 2.3). The tapered regimen involves gradually decreasing the vancomycin over a period of weeks as follows: 125mg four times daily for one week, 125mg three times daily for one week, 125mg twice daily for one week, 125mg daily for one week

followed by 125mg every three days for one week (32). Patients who received a tapered dose of vancomycin seem to have lower recurrence rates compared to standard regimens (65).

For a third recurrence following a tapered regimen, the ACG and ESCMID recommend faecal microbiota transplant (FMT) plus vancomycin (65, 70) (Table 2.3). FMT has been proven to be an effective treatment for RCDI (58, 84) and when compared to other therapies, has the highest rate of success (65, 85). FMT restores the gastrointestinal flora in patients with RCDI where the microbiome has been disturbed and there is decreased microbiological diversity following treatment with antibiotics (58, 84). FMT involves administering faecal material from a healthy donor to a patient's gastrointestinal tract to restore normal intestinal flora (58, 84). This can be done in a number of ways such as via nasogastric or nasojejenal tubes, endoscopy, colonoscopy or enemas (58). Thus far only a single prospective randomised control trial conducted in the Netherlands found that FMT was significantly more effective in the treatment of RCDI when compared to vancomycin (86). Van Nood et al reported a 81% resolution of diarrhoea following a single duodenal infusion of donor faeces (86). Side effects following FMT are minor and include diarrhoea, cramping and belching, all of which resolved within three hours (86). Further research is needed but this new strategy of treating RCDI seems to hold immense promise in particular for chronically relapsing patients.

Table 2.6 Recommendations from the ACG and ESCMID for the management of recurrent CDI

	ACG guidelines (2013)(65)	**ESCMID guidelines (2014)**(70)
First recurrence	Repeat antibiotic given for initial episode using standard regimen (vancomycin or metronidazole)	Vancomycin, Metronidazole or fidaxomicin using standard regimen
Second recurrence	Pulsed vancomycin regimen	Pulsed vancomycin regimen or standard fidaxomicin regimen
Third recurrence	Faecal microbiota transplant plus vancomycin	Faecal microbiota transplant plus vancomycin

2.8.6 Empiric Treatment

Metronidazole and vancomycin are both effective treatments for CDI however the evidence in the literature regarding the benefit of empiric treatment is unknown. A single study published in 2003 by Vasa et al (15) on the effectiveness of empiric metronidazole for presumed CDI found that only 25% of patients with diarrhoea who were treated empirically with metronidazole eventually proved to be positive for *Clostridium difficile.* Patients who were negative for *Clostridium difficile* that were treated empirically received no clinical benefit (15).

It did however show that patients who indeed had CDI significantly benefited from empiric therapy (15). They showed a significantly faster resolution of symptoms; three days compared to 4.2 days in CDI negative patients (15). The authors recommended that empiric treatment be reserved for high risk patients who were unable to tolerate diarrhoea whether it is due to haemodynamic instability or other reasons (15).

The ACG recommends that if a patient has a strong pre-test suspicion for CDI, empiric treatment should be initiated regardless of the laboratory result because the negative predictive values for CDI in these patients are not high enough to exclude disease (65).

ICU patients are at increased risk for contracting CDI as well as developing severe or complicated disease that can result in longer length of stay, increased morbidity and mortality. Increased length of stay especially in ICU adds to the financial burden and negatively impacts resources in the health care system. This is especially true in a country such as South Africa where specialised services such as critical care are extremely limited. As such empiric therapy in the high risk setting of ICU may be a reasonable course of action.

2.8.7 Surgical management

Indications

The vast majority of patients with CDI will respond to appropriate antibiotic management, however 3 to 8% of patients will progress to fulminant colitis that carries a mortality rate of 30 to 90% (1, 56). Only 0.17 to 3.5% of all patients with CDI will require surgical intervention (1).

All patients with severe disease, those who fail to respond to appropriate antibiotic therapy and those who exhibit signs of systemic toxicity will require early surgical consultation (1, 56, 87). Severe disease with lack of response to appropriate antibiotic therapy or clinical deterioration within 24 to 72 hours of initiating maximum therapy is an indication for emergency surgical intervention (45, 56). Other indications for surgery include toxic megacolon, intestinal perforation, signs of organ failure, shock, the need for vasopressors, worsening CT findings and peritonitis (45, 56). Patients who benefit especially from early colectomy are the elderly and patients with raised white cell counts and lactate levels (56, 88).

Colectomy with end ileostomy

Total colectomy with end ileostomy is the procedure of choice in most cases of fulminant colitis as it has slightly lower mortality and morbidity rates compared to segmental resections (1, 89-91). Segmental colectomy is not performed because CDI affects the entire colon and can result in persistent infection (92). Subtotal or total colectomy can result in marked long term morbidity (1). Despite surgical intervention the postoperative mortality rate for fulminant colitis remains extremely high (1, 89, 90). Olivas et al (93) reported on factors contributing to the high surgical mortality that included delay in surgical intervention up to the point where it becomes futile, poor patient selection due to a lack of clearly defined criteria for operative intervention and difficulty in predicting clinical course in CDI. It has however been shown that surgical intervention for fulminant colitis within 48 hours of failure to respond to appropriate antibiotic therapy can improve mortality rates in high risk or severe cases (94, 95) .

Loop ileostomy with vancomycin instillation

Alternative to total colectomy with end ileostomy is the minimally invasive procedure where a loop ileostomy is created with intraoperative colonic lavage with warmed polyethylene glycol solution via the ileostomy, followed by antegrade vancomycin instillation postoperatively (56, 96). In a small study conducted by Neal et al (96)t 42 patients underwent the above mentioned procedure and had reduced mortality compared to traditional total colectomy patients. In this study they also attained colon preservation in 93% of patients (96). Due to the fact that this study was performed at a single centre in a small group of patients, data regarding the broad implementation of this technique is limited.

2.9 Infection control and prevention

2.9.1 Transmission and spread

The major sources of *Clostridium difficile* contamination in hospitals are patients with clinical CDI as well as asymptomatic carriers (97). Patients with CDI with diarrhoea have a much higher environmental contamination when compared to asymptomatic carriers (98). Spores can survive on surfaces for years (1). McFarland et al (98) analysed contamination with *Clostridium difficile* spores in hospital rooms where patients were *Clostridium difficile* negative and found a contamination rate of 8%, proving that spores persist despite routine decontamination of rooms. Quaternary ammonium based disinfectants that are usually used for decontamination of patient rooms are not sporicidal (4). During a CDI outbreak it is recommended that hypochlorite based disinfectants be used (4).

Clostridium difficile is transmitted as spores via the faecal-oral route (4). Once spores are transmitted to a susceptible host they are converted to a vegetative form that replicates and produces toxins that results in clinical disease (5). Spores are transmitted from the contaminated environment to the hands of health care workers. A study conducted by McFarland et al (98) showed that 59% of health care workers caring for patients that were positive for CDI had positive *Clostridium difficile* cultures from their hands. Another suggested mechanism of transmission is direct inoculation of spores into bowel by contaminated rectal thermometers (97).

Factors that contribute to the transmission of *Clostridium difficile* include resistance of spores to frequently used antiseptics and disinfectants, indiscriminate antibiotic usage, failure to recognise or diagnose patients infected with *Clostridium difficile* and re-admission of patients with CDI (97). A major problem in preventing *Clostridium difficile* transmission is that even though alcohol based hand sanitizers are effective against the vegetative form of *Clostridium difficile,* the spores are resistant. Sanitisers do not reduce the number of viable spores (32). Only washing hands with soap and water has been shown to remove spores from hands (32).

2.9.2 Prevention of spread

Infection control strategies in ICU exist on a continuum and include vertical and horizontal strategies (27). Vertical strategies aim to reduce colonisation or infection of a specific organism whilst horizontal interventions aim to reduce the spread of general pathogens by using universal measures such as hand washing and gloves (27).

Preventing the acquisition of infection and spread of CDI by indirect or direct contact with spores requires a multi-facetted approach. Due to the fact that there is no effective vaccine, infection control measures emphasizes prevention of spread and antibiotic stewardship (32). Prevention of spread can be divided into different strategies namely early detection, hand hygiene, barrier and contact precautions, disposable equipment and environmental decontamination.

Early detection

Early recognition of CDI enables earlier treatment and implementation of infection control measures. All non-cultured based tests for *Clostridium difficile* have been validated in symptomatic patients only and it is believed that sensitivity, specificity and positive predictive value of most assays are lower in asymptomatic patients resulting in false positives (99). Thus it is not recommended to screen asymptomatic patients for CDI. A high index of suspicion for all patients with diarrhoea and risk factors for CDI such as recent antibiotic use or hospitalisation, age over 65 years and chemotherapy should prompt early testing (65, 99). Diagnostic tests with a fast turnaround time, high sensitivity and specificity should be used to expedite faster results.

A hospital based infection control program can decrease the incidence of CDI (65). Infection control programs ensure that correct staff members are immediately informed of positive *Clostridium difficile* tests to initiate early treatment and appropriate contact precautions.

Hand hygiene and barrier precautions

Hand hygiene is the cornerstone of prevention and spread of *Clostridium difficile.* Alcohol based hand gels are effective against vegetative cells but spores are resistant (1). A study found that up to 30% of spores remain on hands following the use of 3 ml of alcohol based hand gel (1). Despite the fact that alcohol based hand gels are not sporicidal the use of these gels are not associated with higher transmission rates (27). Washing hands with soap and water is proven to be superior in removing *Clostridium difficile* from hands and it is the recommended method of hand hygiene when CDI is suspected or confirmed (100).

Basic infection control strategies such as the use of gloves have been proven to be effective in decreasing the spread of *Clostridium difficile* (5, 101). A study conducted in 1990 showed a six-fold decrease in the incidence of CDI when vinyl gloves were used for all body contact with patients (5, 101). The evidence for the use of gowns in combating the spread of *Clostridium difficile* is not as well documented but it is still recommended (65).

Ideally patients with CDI should be placed in an isolation room, if this is not possible CDI patients should be grouped together to prevent transmission to other patients (64). Mcfarland et al (98) found higher rates of infection in double rooms as opposed to single rooms as well as an increased risk of contracting CDI following exposure to a roommate with a positive *Clostridium difficile* culture.

Disposable equipment

Single use disposable equipment should be used for patients with CDI wherever possible (65). In several studies replacing electronic rectal thermometers with disposable thermometers has resulted in a reduction in the incidence of CDI (63, 65). Non-disposable equipment should be used exclusively in the infected patient's room and cleaned properly following use (65).

Environmental decontamination

Clostridium difficile spores can remain viable on surfaces for months or years as they are resistant to desiccation (63, 98). It has been proven that *Clostridium difficile* is present on healthcare professionals' hands and that the degree of colonization correlates with the extent of environmental contamination (102). The degree of contamination is increased by colonised patients and patients with diarrhoea (102).

Environmental decontamination decreases the incidence of CDI (63, 103). Quaternary ammonium-based disinfectants that are routinely used for environmental cleaning in hospital are not sporicidal and may in fact aid sporulation (64, 104). Numerous detergents are effective in killing the vegetative form of *Clostridium difficile* but only chlorine-based disinfectants and vaporised hydrogen peroxide have been shown to be sporicidal (64). Although vaporised hydrogen peroxide is effective in killing spores it is expensive and impractical (104). Unbuffered hypochlorite solution has been proven to decrease environmental contamination and the incidence of CDI (65, 103). A chlorine-containing detergent with a minimum concentration of 5000 parts per million of chlorine should be used for at least 10 minutes in areas with a high

incidence of CDI as a practical and affordable intervention that has been proven to be effective (65, 103).

Antibiotic stewardship

Exposure to antibiotics is the most important risk factor for the development of CDI (1, 105). The greater the number, higher dose and longer duration of antibiotics, the greater the risk of developing CDI (44, 104). In addition, certain classes of antibiotics confer greater risk. The third generation cephalosporins carry the highest for the development of CDI but almost all antibiotic classes have been associated (44, 45). Cephalosporins, clindamycin and fluoroquinolones are frequently associated with CDI while penicillins, macrolides and sulphonamides/ trimethoprim are associated to a lesser degree (46, 47). The exception is that the tetracyclines are not associated with CDI and seem to be protective against contracting nosocomial CDI (47). Fluoroquinolones are moderately associated with the NAP1/BI/027 strain responsible for the outbreaks in Europe, Canada and America (44, 104). As such, their use should be restricted in areas that have a high incidence of the NAP1/BI/027 strain (5).

Antimicrobial stewardship encourages the judicious use of antibiotics with the aim to improve patient outcome, decrease antibiotic resistance and decrease expenditure (27). Reducing antimicrobial use has been proven to decrease the incidence of nosocomial CDI (32). Programs that focus specifically on reducing the use of high risk antibiotics such as cephalosporins, fluoroquinolones and clindamycin have been effective in decreasing the incidence of CDI (44, 45, 65). A Cochrane systematic review published in 2013 showed that implementing antibiotic stewardship programs that changed antibiotic prescribing practices were safe, reduced antibiotic resistance, nosocomial infection and the incidence of CDI (27, 106).

Probiotics

Evidence for the use of probiotics for the prevention of CDI is conflicting. Antibiotics disrupt the intestinal microbiome resulting in greater susceptibility for CDI (58). Theoretically, therapy that prevents disturbance of intestinal flora will reduce the likelihood of CDI (58).

Early studies indicated that the use of probiotics such as lactobacillus decreased the incidence of antibiotic associated diarrhoea and CDI however most of these were under powered for prevention of CDI (32). A recent Cochrane review that included 23 randomised control trials and 4213 patients found moderate quality evidence suggesting that probiotics are safe and effective

in the prevention of CDI (107). In contrast, a recent large randomised, double blind, placebo-controlled multicenter trial that enrolled 17 420 patients found no evidence that probiotics (a multistrain preparation of lactobacilli and bifidobacteria) was effective in preventing CDI or antibiotic associated diarrhoea (108).

Overall, the evidence for the use of probiotics for treatment or prevention of CDI is not sufficient and their use is not recommended (32, 45, 58). However, probiotics are inexpensive and do not have significant side effects and despite the lack of evidence for efficacy they are often prescribed in an attempt to treat or prevent CDI (58).

2.10 Conclusion

In this chapter the literature review was presented. The following topics were discussed: etiology and epidemiology of *Clostridium difficile,* pathophysiology, NAP1/BI/027 strain, risk factors, clinical features, diagnosis, treatment as well as infection control and prevention. The following chapter, chapter 3, the research methodology will be discussed.

Chapter 3: Research methodology

3.1 Introduction

In this chapter the problem statement, aims and objectives, ethical considerations, research methodology, validity and reliability of this study will be discussed.

3.2 Problem statement

Clostridium difficile is not only the leading causal pathogen of antibiotic associated diarrhoea (3) but also the most common causal pathogen for nosocomial infectious diarrhoea (4, 5). ICU patients with CDI carry a higher mortality rate and increased length of ICU and hospital stay (14). Emergence of the more virulent strain in the United States, Canada and Europe has increased both incidence and severity of CDI (10). Empiric therapy, in patients discovered later to have CDI, was shown to be beneficial by shortening the time to resolution of symptoms whilst CDI negative patients gained no benefit (15). South African data regarding CDI are limited. Institutional knowledge regarding CDI occurrence has the potential to improve management of CDI. The positive yield and occurrence of NAP1 strain in stool samples sent for *Clostridium difficile* testing from 576 ICU and 579 HCU at CMJAH was not known. Whether patients with suspected CDI are treated empirically or not was also not known.

3.3 Aims and objectives

3.3.1 Aim

The aim of this study is to determine the positive yield and number of NAP1 strains of all stool samples sent for *Clostridium difficile* testing from 576 ICU and 579 HCU at CMJAH from 1 January 2014 until 31 December 2015.

3.3.2 Objectives

The objectives of this study were to:

- determine the positive yield of *Clostridium difficile* in stool samples sent from 576 ICU and 579 HCU at CMJAH from 1 January 2014 until 31 December 2015
- determine the number of stool samples that were positive for *Clostridium difficile* out of the total number of patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015
- determine the number of NAP1 positive samples
- estimate the time from onset of diarrhoea until the time the stool sample was received in the laboratory
- estimate the time from sample received in the laboratory until laboratory diagnosis
- estimate the time from onset of diarrhoea to initiation of treatment
- determine whether the patient was treated empirically or following laboratory diagnosis
- determine whether a patient was started on metronidazole, vancomycin or both
- determine whether APACHE II score differs between a positive and negative *Clostridium difficile* result

3.4 Ethical considerations

Approval to conduct this study was granted by the Postgraduate Committee (Appendix A) as well as the Human Research Ethics Committee (Medical) of the University of Witwatersrand (Appendix B). Furthermore approval was obtained from the CEO of CMJAH (Appendix C) as well as the Head of Department of Anaesthesiology (Appendix D). Permission to access patient records was obtained from the Director of ICU and 579 HCU (Appendix E). Permission to use the National Health Laboratory Services (NHLS) database was given by the NHLS database gatekeeper (Appendix F).

Data was collected anonymously. Confidentiality of patients was ensured as only the researcher and supervisors has access to the raw data. The data collected will be stored securely for five years. Every patient was a data identification number. The data identification number and hospital number of each patient included in the study were entered on a sheet that was kept

separate from the data collection sheet (Appendix E). Only the data identification number was used for data analysis.

This study was retrospective and did not impact clinical management of patients as it did not involve any therapeutic interventions.

This study was conducted in accordance with the Declaration of Helsinki (18) and the South African Good Clinical Practice Guidelines (19).

3.5 Research methodology

3.5.1 Research design

A retrospective, descriptive, contextual study design was followed in this study.

Retrospective studies investigate phenomena or problems that occurred in the past and utilise data available for a certain period (109). Clinical records for the year 2014 and 2015 were used for data collection in this study.

A descriptive study aims to provide information from a representative sample in a certain group or population, in an area where data is still lacking, without necessarily establishing a link between cause and effect (110). This study described the occurrence of *Clostridium difficile* positive samples and NAP1 strains in ICU and 579 HCU. It also described the time intervals between onset of diarrhoea, laboratory diagnosis and treatment.

A contextual study is a study that is conducted in a specific group or population. De Vos et al describe this context as a “small scale world” (111). For this study the small scale world was 576 ICU and 579 HCU at CMJAH.

3.5.2 Study population

The study population was the clinical records of patients admitted to 576 ICU and 579 HCU for whom stool samples were sent for *Clostridium difficile* test.

3.5.3 Study sample

Sample size

The sample size was determined by the number of available clinical records for the year 2014 and 2015.

Sample Method

Consecutive convenience sampling was used to assess clinical records. Convenience sampling assesses the most readily available individuals for the study (110). Consecutive convenience sampling is a subtype of convenience sampling where every available individual in the study population is selected and is the best manner in which to conduct non-random sampling (112).

Inclusion and exclusion criteria

Inclusion criteria for this study were:

- The clinical records of all patients over the age of 18 years admitted to 576 ICU or 579 HCU who had stool samples sent for laboratory testing for *Clostridium difficile*

Exclusion criteria for this study were:

- Illegible or incomplete records
- In the case were a single patient had more than one sample sent for the same type of test and all results remained the same, only the first sample sent to the laboratory for that particular test was used to estimate the time from onset of diarrhoea until the time the stool sample was received in the laboratory. Similarly only the first sample sent was used to estimate the time from onset of diarrhoea to treatment and whether the patient was treated empirically or following laboratory diagnosis

3. 5. 4 Data collection

Data collected

A data collection sheet containing key elements as identified from the literature review was drawn up. The following parameters were recorded:

- Age
- APACHE score
- Clostridium difficile positive/negative
- NAP1 positive/negative
- Number of days in 576 ICU or 579 HCU at onset of diarrhoea
- Time from onset of diarrhoea to sample received in laboratory
- Time from onset of diarrhoea to laboratory diagnosis
- Time from onset of diarrhoea to treatment
- Empiric treatment: yes/no
- Treatment:
    - Oral metronidazole
    - Intravenous metronidazole
    - Oral vancomycin
    - Oral and intravenous metronidazole

Data collection process

All samples sent to the NHLS on an inpatient basis include the patient's ward number on the laboratory requisition form. Following a formal data request, the NHLS provided a list of stool samples as well as results that were sent from 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015. Laboratory results were reviewed and data was captured onto the data collection sheet. The relevant patient's ICU chart was obtained and information collected and captured with the aid of the data collection sheet.

3.5.5 Data analysis

Microsoft Excel 2007 was used to create the data collection sheet and capture the relevant data. Descriptive and inferential statistics were used. Categorical variables were described using frequencies and percentages. Statistical analysis was done in consultation with a biostatistician. The Mann U Whitney test was used to determine significance and medians in all nonparametric data, in which case a p-value of <0.05 was considered significant.

3.6 Validity and reliability of the study

According to Botma et al (113), validity is the degree to which measurements represent the true value. In order to ensure validity the appropriate study design and data collection techniques should be used (113).

Reliability refers to the concept of reproducibility of measurement (113).

Validity and reliability in this study was ensured by the following:

- The study design was developed after an extensive literature review
- Data was collected by a single researcher
- Data collection sheets were developed in conjunction with experts in the field namely an intensivist as well as the director of ICU

3.7 Conclusion

In this chapter the problem statement, aims and objectives, ethical considerations, research methodology and reliability and reliability of this study were discussed. In the following chapter, chapter 4, the results of the study will be discussed.

Chapter 4: Results and discussion

4.1 Introduction

In this chapter the sample realisation, results according to the objectives and a discussion regarding the results are presented.

4.2 Sample realisation

Following a data request the NHLS provided a list of stool samples that were sent for *Clostridium difficile* testing from 576 ICU and 579 HCU for the period from 1 January 2014 until 31 December 2015. It included a total of 293 stools samples from 192 patients. Of the 293, 10 samples from five patients were excluded as the patients were under the age of 18 years. Thus a total of 283 samples from 187 patients were included in this study. A breakdown of the samples is presented in figure 4.1 and 4.2.

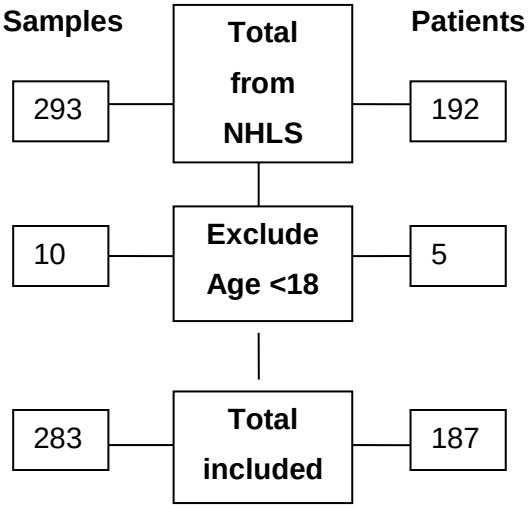

Figure 4.1 NHLS data breakdown

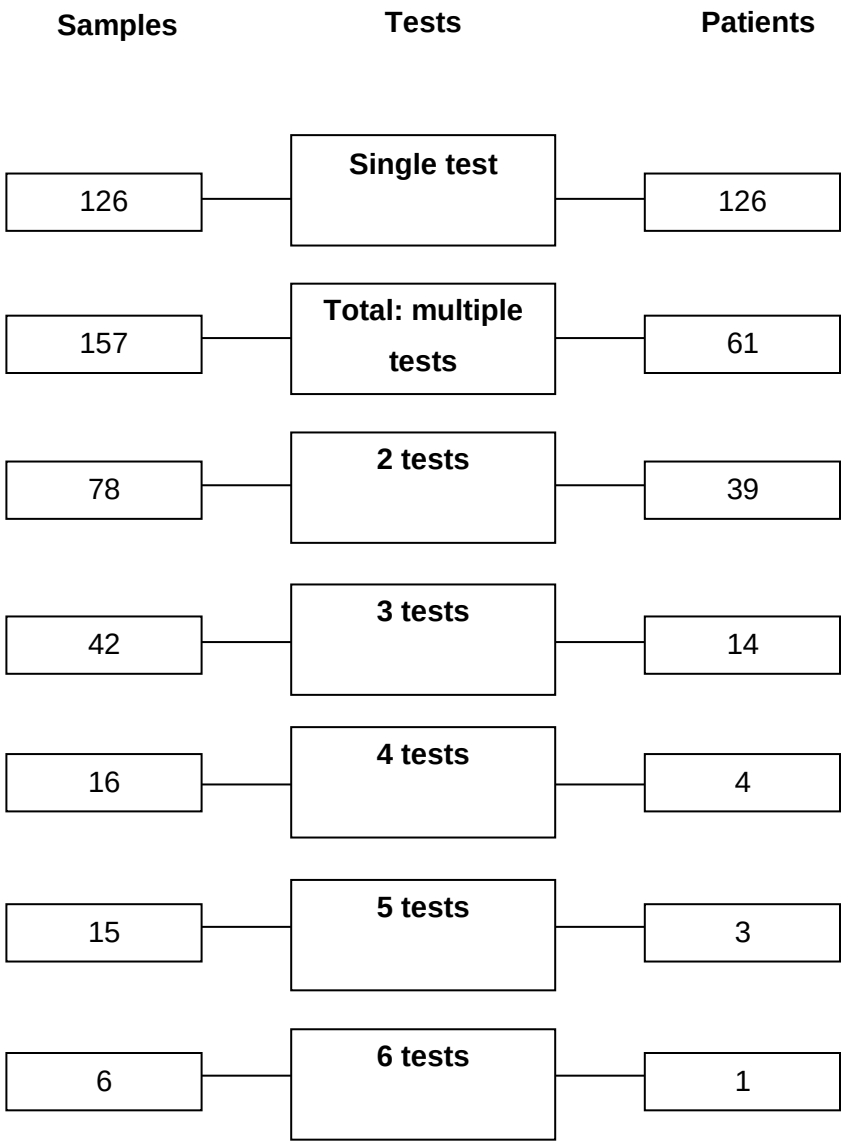

Figure 4.2 NHLS sample breakdown

Following a review of the clinical records of the possible 187 eligible patients only 128 clinical records (179 samples) were available and complete. In this study 59 (31.55%) clinical records were excluded as they were not found or the records were incomplete.

In such cases where a patient had more than one test sent for *Clostridium difficile* and the result remained negative for all subsequent tests only the time from the first sample sent was used in all objectives related to time. If however the diagnosis changed from negative to positive each sample would be used. Only two patients had samples where the initial result was negative but subsequently changed to positive after five and 14 days respectively. In only one case a subsequent sample was sent 16 days after a positive *Clostridium difficile* test and the sample was negative. In this case the second sample was not included as testing for cure is not recommended (65).

4.3 Results

4.3.1 Patient demographics

Of the 187 patients included in this study 101 (54.01%) were female and 86 (45.99%) were male (Figure 4.3). Age ranged from 18 years to 83 years with a median age of 40 years. 128 clinical records were available for review, of these 87 (67.97%) was admitted to 576 ICU or 579 HCU with a medical diagnosis and 41 (32.03%) were admitted with a surgical diagnosis. The amount of days spent in ICU prior to onset of diarrhoea ranged from 0 to 22, with a median of three days.

4.3.2 Determining the positive yield of *Clostridium difficile* in stool samples sent from 576 ICU and 579 HCU

A total number of 283 samples from 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015 were included in this study. Of these samples 38 (13.42%) tested positive for *Clostridium difficile* (Figure 4.3).

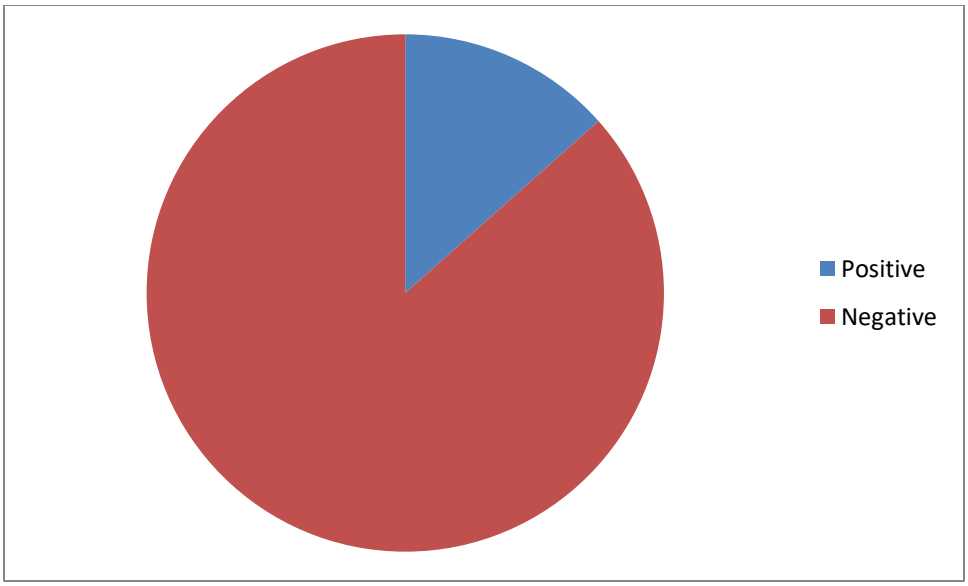

Figure 4.3 Positive yield of *Clostridium difficile*

4.3.3 Determining the number of stool samples that are positive for *Clostridium difficile* out of the total number of patients admitted in 576 ICU and 579 HCU

Out of the total number of 3941 patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015, 38 (0.96%) samples from 26 individual patients (0.66%) sent for *Clostridium difficile* tests were positive.

4.3.4 Determining the number of NAP1 positive samples

The NHLS does not test for the NAP1/BI/027 strain and thus we were unable to determine the number of NAP1/BI/027 positive samples. When a sample is sent for PCR for toxigenic *Clostridium difficile,* the NAP1/BI/027 is reported as 'presumptive negative'.

4.3.5 Estimating the time from onset of diarrhoea until the time the stool specimen was received in the laboratory

139 clinical records were reviewed and found to have documented time of onset of diarrhoea.

The time from onset of diarrhoea until a stool sample was received in the laboratory ranged from zero days (same day) to 61 days. The median time was two days. The data is shown in the frequency histogram figure 4.4.

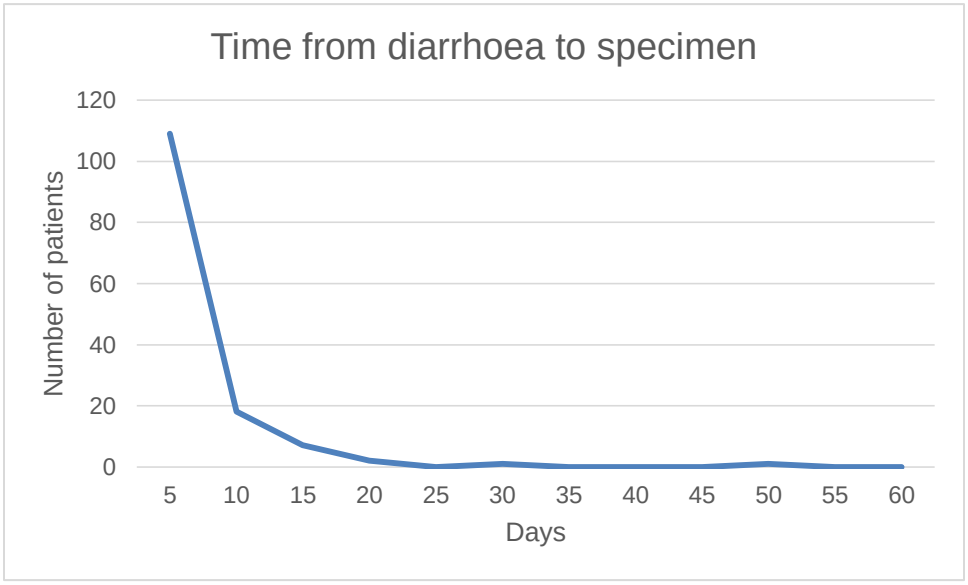

Figure 4.4 Time from onset of diarrhoea to specimen received at the laboratory

4.3.6 Estimating the time from sample received in the laboratory until laboratory diagnosis

To estimate the time from the sample received to diagnosis, in other words the lab turnaround time, 165 samples for *Clostridium difficile* PCR had sufficient data to be used. The median time for *Clostridium difficile* PCR was five hours.

To estimate the time from sample received in the laboratory until the time to diagnosis for *Clostridium difficile* toxin, 100 samples had sufficient data to be used. Six samples were excluded as the time taken was more than seven days and this was considered to be an error

and as such true outliers. The median time taken for *Clostridium difficile* toxin test to be performed was 23 hours.

A Mann-Whitney U test was performed and results showed a significant difference ($p<0.0001$) in the time taken for toxin EIA compared to the PCR.

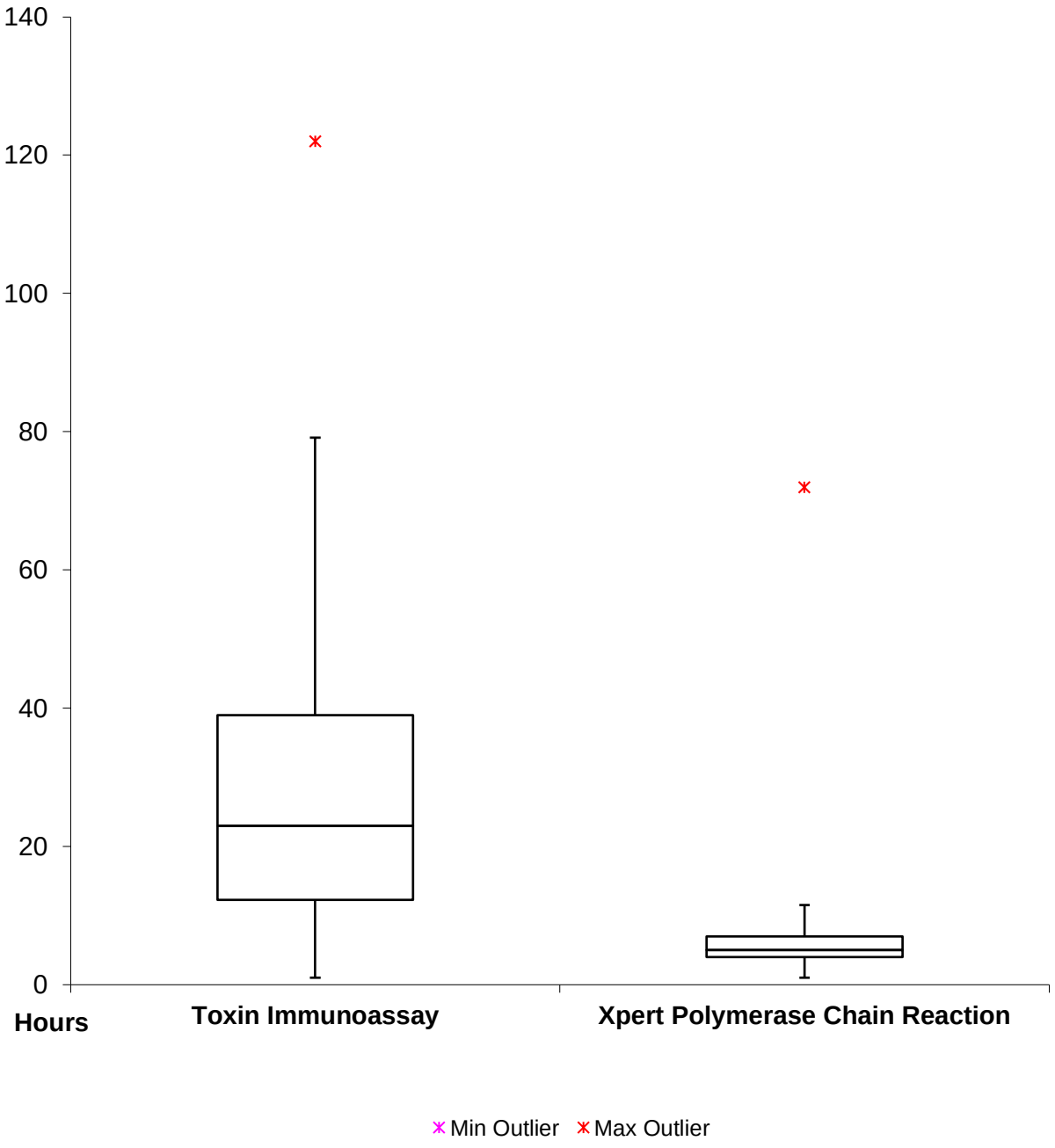

Figure 4.5 Comparison between toxin EIA and PCR turnaround time

4.3.7 Estimating the time from onset of diarrhoea to initiation of treatment

47 clinical records reviewed had sufficient data to determine time from onset of diarrhoea until the time treatment was initiated. The median time was 40 hours.

4.3.8 Determining whether the patient was treated empirically or following laboratory diagnosis

In eight of the clinical records it was not possible to determine whether treatment was started empirically or not as data was missing or the patient was discharged from the unit prior to treatment initiation. Of the remaining 120 ICU charts available for review, 24 patients (20%) received empiric treatment (Figure 4.6).

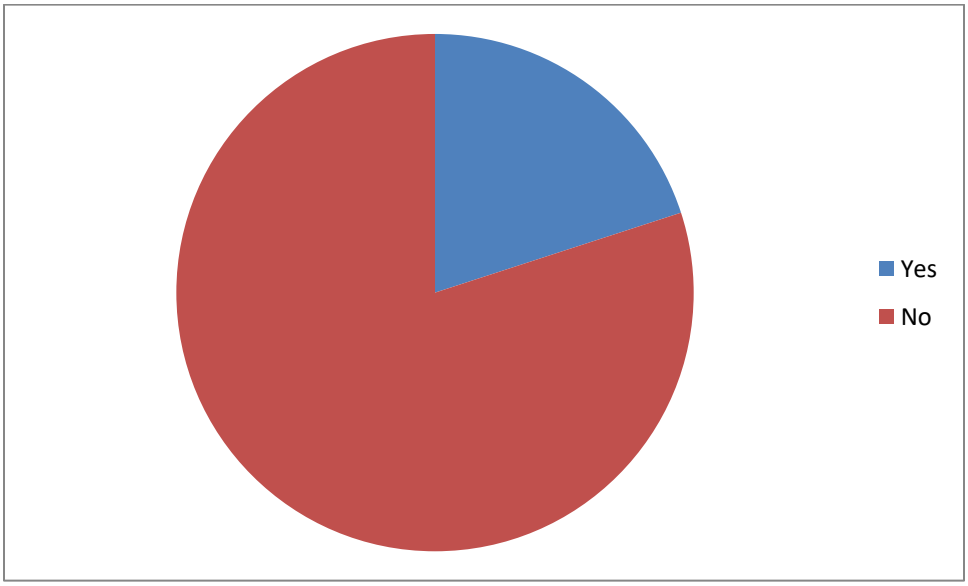

Figure 4.6 Empiric treatment

4.3.9 Determining whether a patient was started on metronidazole, vancomycin or both

In eight of the clinical records it was not possible to determine whether treatment was started or not as data was missing or the patient was discharged from the unit prior to treatment initiation. In 576 ICU and 579 HCU all patients received metronidazole (500mg) three times daily if oral treatment was given and metronidazole (400mg) three times daily if intravenous treatment was given. No treatment was initiated in 77 (64.17%) of patients. Of the remaining 43 patients who were started on treatment, 10 (23.33%) were started on oral metronidazole only, two (4.65%) were started on intravenous metronidazole only and 28 (65.12%) were started on both oral and intravenous metronidazole (Figure 4.7). Only two (1.67%) had received oral vancomycin and in both these cases the patients also received both oral and intravenous metronidazole. One of these patients had presented to ICU post colectomy for toxic megacolon secondary to CDI. This patient received vancomycin (250mg) orally four times per day per nasogastric tube. The second patient received the standard dose of vancomycin (125mg) four times per day orally.

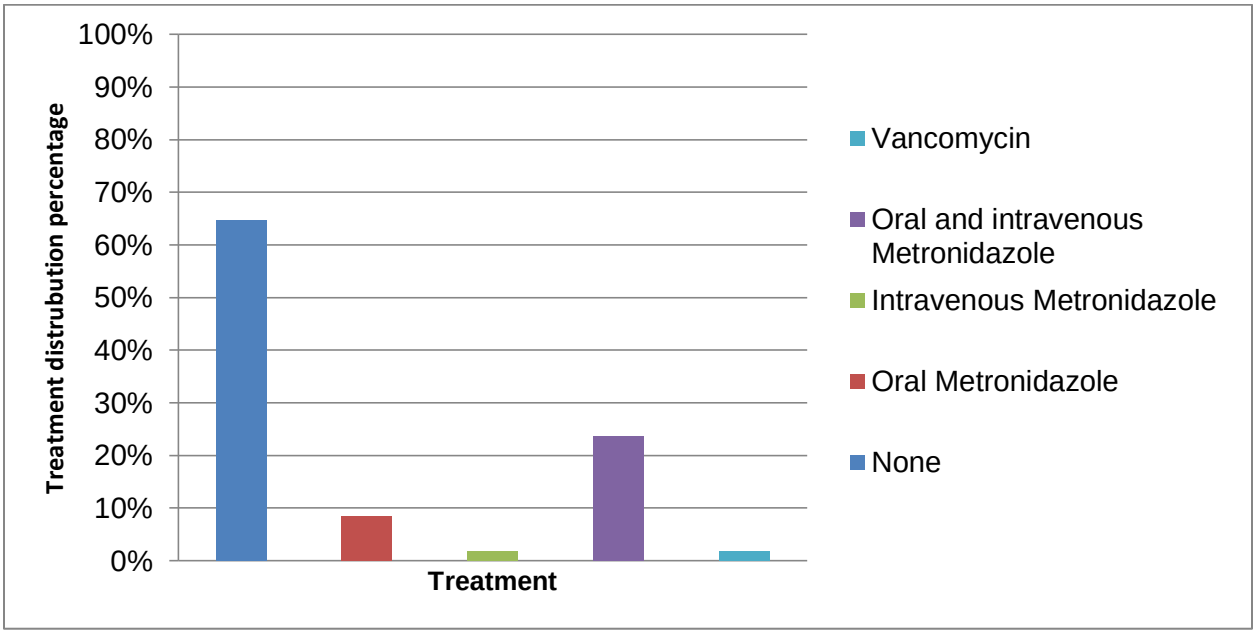

Figure 4.7 576 ICU and 579 Treatment distributions

4.3.10 Determining whether APACHE II score differs between a positive and negative *Clostridium difficile* result

From the 128 ICU records to review 118 (19 *Clostridium difficile* positive patients and 99 negative) had sufficient data to calculate the APACHE II scores. The median APACHE II score for CDI negative patients was three with a range of 0-22. The median APACHE II score for CDI positive patients was 22 with a range of 9-37. A Mann- Whitney test was done and found a $p < 0.0001$. Thus the APACHE II scores for patients who tested positive for *Clostridium difficile* were significantly higher than those who tested negative.

4.4 Discussion

4.4.1 Introduction

CDI is the most common causal pathogen for nosocomial infectious diarrhoea as well as for antibiotic associated diarrhoea (3, 4). Whilst most nosocomial infections have declined since 2001 the incidence of CDI has increased (1, 6). The increased incidence can be attributed to the widespread use of broad spectrum antibiotics (3, 7). ICU patients are at higher risk for contracting CDI as well as at increased risk for developing complicated disease. Changes in epidemiology and emergence of the more virulent strains should prompt us to be more vigilant and more aware of *Clostridium difficile* in our institution.

4.4.2 CDI in ICU

Clostridium difficile is the most important cause of infectious nosocomial diarrhoea from an epidemiological view (13). A systematic review conducted by Karanika et al (14) that included 22 studies and 80 835 ICU patients found that 2% of ICU patients contracted CDI whilst in ICU. Incidence of CDI in ICU in the literature ranges from 0.3-3% (12, 14, 25, 26). In this study out of the total number of 3941patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015, 38 (0.96%) samples from 26 individual patients (0.66%) sent for *Clostridium difficile* tests were positive. This number does not reflect the true incidence of CDI in ICU and 579 HCU at CMJAH as not all patients with diarrhoea in ICU were included in this study, only

those who had samples sent for *Clostridium difficile* tests but can serve as an estimate of the incidence in the absence of more accurate data.

In this study 38 (13.42%) out of 283 samples or 26 patients out of 187 (13.90%) sent to the laboratory tested positive for *Clostridium difficile.* In ICU 9.3-11% of patients who develop diarrhoea are diagnosed with CDI (12, 14). If we assume that all patients in ICU who develop diarrhoea have a stool sample sent to the laboratory for microscopy and *Clostridium difficile* testing our proportion of ICU patients with CDI is slightly higher compared to the literature. This can be explained by differences in exclusion criteria as most studies exclude patients who develop diarrhoea in the first 24 hours following admission to ensure it is truly ICU acquired diarrhoea where as in this study we did not.

In this study the median onset of diarrhoea was on the third day following admission. Ang et al (114) reported acquisition of CDI in ICU on the seventh day following admission. Thibault et al (26) reported the median onset of diarrhoea in ICU on day six following admission. The differences in median days of onset of diarrhoea can possibly be explained by the studies' exclusion criteria. In the study conducted by Ang et al (114) patients that acquired CDI prior to ICU admission was excluded from the study. In the study conducted by Thibault et al (26) patients that developed diarrhoea in the first 24 hours of admission to ICU where excluded to ensure the diarrhoea was ICU acquired. In our study we did not make the distinction whether CDI was ICU acquired or acquired prior to admission to ICU. Another possible reason for the earlier onset of diarrhoea in this study might reflect a longer stay in hospital prior to admission to ICU when compared to international studies where admission to ICU might be considerably sooner.

4.4.3 NAP1/BI/027

In this study we were not able to determine the number of NAP1/BI/027 positive samples due to the fact that the NHLS does not test for this strain currently. This might be because the incidence of this toxigenic strain is so low it is not financially feasible to test for it. It appears that the incidence of the NAP1/BI/027 strain is very rare in South Africa but data is very limited.

In 2013 Nana (23) did a prospective study on laboratory confined CDI at CMJAH. 190 samples were included in the study and 43 were found to be toxigenic culture positive (23). Not one of

the 43 toxigenic culture positive were found to have the *tcdC* gene deletion associated with the 027 ribotype (23). A prospective study done at Groote Schuur Hospital by Rajabally et al (11) over a period of 15 months found that out of the 643 patients included in this study only two cases of the NAP1 strain were reported . Samie et al (12) reported on PCR detection of Clostridium difficile in Vhembe district in South Africa. The study included 322 stool samples from hospital in-patients and school children and reported the molecular characterization of *Clostridium difficile* in their district (22). The binary toxin in isolation was only found in three of the samples (22).

4.4.4 Time from onset of diarrhoea to treatment

The time from onset of diarrhoea until a stool sample was received in the laboratory ranged from 0 days (same day) to 61 days. The median time was two days in this study. The very long period of 61 days prior to a sample being sent to the laboratory can be explained by a number of reasons. The patient might have presented to ICU with pre-existing chronic diarrhoea and a sample sent only after the frequency or nature of diarrhoea changed. The possibility also exists that there was an error at some point in the process that could include: failure to collect a specimen, insufficient or rejected sample, incorrectly labelled sample or sample could have been misplaced or lost. Currently neither ACG nor ESCMID have recommendations regarding the length of time of diarrhoea prior to testing for CDI. Both recommend only testing stool from patients with diarrhoea and not to test asymptomatic patients due to high rates of asymptomatic colonisation (65, 70).

The median time from sample received in the laboratory until diagnosis was reported for *Clostridium difficile* PCR was five hours. The median time from sample received in laboratory until diagnosis for the toxin EIA was 23 hours. The time difference between the two tests was significant ($p<0.0001$). There are some data to support that faster diagnosis can lead to reduction in CDI complications such as admission to ICU, colectomy and death (58). Furthermore earlier diagnosis results in a reduction in duration of empiric antibiotic therapy and isolation precautions (59). Although most laboratories use toxin EIA as a diagnostic test, at present EIA for toxins is not considered sensitive enough to be used as a stand-alone diagnostic test (58). PCR tests are more sensitive and have faster turnaround times (23). The drawback is the higher cost of PCR and occurrence of false positives in asymptomatic carriers.

The median time from onset of diarrhoea to initiation of treatment was 40 hours with a range of one to 280 hours. The wide time range can possibly be explained by the fact that symptoms do not develop uniformly in all patients. Symptoms like diarrhoea may start during antibiotic therapy, five to 10 days following cessation of antibiotic therapy or less commonly symptoms can present as late as 10 weeks after initial antibiotic treatment (115). In addition to varying presentation of diarrhoea treating physicians might elect to only start treatment once the diagnosis of CDI has been confirmed.

4.4.5 Empiric treatment

In this study 24 patients (20%) received empiric treatment. The evidence in the literature regarding the benefit of empiric treatment is unknown. A single study published in 2003 by Vasa et al (15) on the effectiveness of empiric metronidazole for presumed CDI found that only 25% of patients with diarrhoea who were treated empirically with metronidazole eventually proved to be positive for *Clostridium difficile.* Patients who were negative for *Clostridium difficile* that were treated empirically received no clinical benefit (15).

It did however show that patients who indeed had CDI significantly benefited from empiric therapy (15). They showed a significantly faster resolution of symptoms; three days compared to 4.2 days in CDI negative patients (15). The authors recommended that empiric treatment be reserved for high risk patients who were unable to tolerate diarrhoea whether it is due to haemodynamic instability or other reasons (15).

Patients in ICU might benefit from empiric therapy when one considers that the prevalence of CDI is significantly higher in ICU than the general hospital population, patients in ICU are exposed to multiple risk factors for CDI, the mortality rate for ICU patients with CDI infection is significantly higher than for those without CDI, patients in ICU have a high risk of adverse events due to complicated CDI, CDI also significantly increases not only ICU but also hospital length of stay (14, 27, 29).

The ACG recommends that if a patient has a strong pre-test suspicion for CDI, empiric treatment should be initiated regardless of laboratory result because the negative predictive values for CDI in these patients are not high enough to exclude disease (65).

4.4.6 Treatment

In this study no treatment was initiated in 77 (64.17%) of patients. Of the remaining 43 patients who were started on treatment 10 (23.26%) were started on oral metronidazole only, 2 (4.65%) were started on intravenous metronidazole only and 28 (65.12%) were started on both oral and intravenous metronidazole. In eight of the clinical records it was not possible to determine whether treatment was started due to incomplete data.

Currently the recommendation for the first episode of mild to moderate CDI is metronidazole (500mg) orally three times daily for 10 days by both the ACG and ESCMID (65, 70). The combination of oral and intravenous metronidazole simultaneously is not currently recommended by the ACG or ESCMID. The ACG only recommends intravenous metronidazole in addition to oral vancomycin in the case of severe disease with significant abdominal distention (65). It seems to be common practice in 576 ICU and 579 HCU to initiate patients with suspected or confirmed CDI on both oral and intravenous metronidazole. Oral metronidazole has been found to be effective in mild to moderate disease resulting in clinical resolution of disease (61). Whether the addition of intravenous metronidazole to oral metronidazole adds any benefit is not known and requires further study**.** The addition of intravenous metronidazole to oral vancomycin however has been shown to reduce mortality (116).

In this study only 2 (4.65%) had received vancomycin PO and in both these cases the patients also received both oral and intravenous metronidazole. The first patient received high dose vancomycin (2g in four divided doses per day) while the second patient received low dose vancomycin (500mg in four divided doses per day). None of the patients in this study received rectal vancomycin. The first of these patients had presented to ICU post colectomy for toxic megacolon secondary to CDI even though this patient had a negative toxin enzyme immunoassay. The second demised in ICU from septic shock. Both patients suffered from severe and complicated disease.

In the presence of severe disease without significant abdominal distension, both ACG and ESCMID recommend the use of oral vancomycin (125mg) four times daily for 10 days (65, 70). For the treatment of severe disease associated with significant ileus or abdominal distention, the ACG recommends the use of vancomycin (500mg) orally and vancomycin (500mg) diluted to

500ml per rectum four times daily (65). In addition, they recommend the addition of metronidazole (500mg) given four times daily intravenously (65). The ESCMID recommends vancomycin (125mg) four times daily for 10 days in this patient group. (70).No significant difference was found in cure rates when high dose vancomycin (2g in 4 four divided doses per day) was compared with low dose vancomycin (500mg in 4 four divided doses per day) (71). Both ACG and ESCMID recommend surgical consultation in severe disease (65, 70).

4.4.7 APACHE II Scores

In this study the median APACHE II score for negative patients was three with a range of 0-22. The median APACHE II score for positive patients was 22 with a range of 9-37. A Mann-Whitney test was done and found a $p < 0.0001$. Thus the APACHE II scores for patients who tested negative for *Clostridium difficile* were significantly lower than those who tested positive.

Thibault (26) found significantly higher APACHE II scores in patients with diarrhoea than those without, whilst Keaneally (29) and Li (52) found that patients with CDI have higher APACHE II scores than those who do not. This study showed similar results. Higher APACHE II scores are considered a risk factor for contracting CDI and increases the risk of mortality (29, 52).

4.4.8 Significance of findings

This study demonstrated a positive yield (13.42%) of samples sent to laboratory for *Clostridium difficile* testing. Taking into account the limitations of this study and assuming that all patients with diarrhoea had a sample sent for *Clostridium difficile* testing, this figure is comparable to the 9.3-11% of patients reported in the literature who develop diarrhoea in ICU and are diagnosed with CDI (12, 14). Working under the same assumption, the positive yield of 0.66% of patients is comparable with the incidence of CDI in ICU in the literature that ranges from 0.3-3% (12, 14, 25, 26).

This study demonstrated a significant difference in turnaround time when comparing the time taken for the EIA for toxin A and B compared to the PCR, 23 hours compared to five hours. This can be used to guide decisions regarding choice of diagnostic tests at our institution. The ideal

diagnostic test for CDI has not yet been established. Testing protocols are determined by availability, cost and turnaround times at different institutions.

We determined that a large proportion of patients (20%) receive empiric treatment. It was also evident that the majority of patients who are initiated on treatment receive both oral and intravenous metronidazole concurrently. This practice is not recommended by the ACG or ESCMID for the treatment of first episode of mild to moderate disease at the moment. This finding can be used to re-evaluate current practice and revise treatment protocols at our institution.

In this study we have found that patients with CDI have a higher APACHE II score than those who do not. This can be used to guide clinical decisions on whether to initiate empiric treatment or to await laboratory diagnosis.

4.5 Conclusion

In this chapter the sample realisation, the results of the study and a discussion regarding the results was presented. In the following chapter, chapter 5, a summary of the study, the limitations of the study, recommendations and the conclusion of the study are discussed.

Chapter 5: Study summary, recommendations and conclusion

5.1 Introduction

In this final chapter a study summary, the limitations of the study, recommendations, and conclusion of the study are discussed.

5.2 Study summary

Whilst most nosocomial infections have declined since 2001 the incidence of CDI has increased (1, 6). *Clostridium difficile* is the most important cause of infectious nosocomial diarrhoea from an epidemiological view (13). ICU patients are at higher risk for contracting CDI as well as at increased risk for developing complicated disease. Changes in epidemiology and emergence of the more virulent strains should prompt us to be more vigilant and more aware of *Clostridium difficile* in our institution.

The aim of this study was to determine the positive yield and number of NAP1 strains of all stool samples sent for *Clostridium difficile* testing from 576 ICU and 579 HCU at CMJAH from 1 January 2014 until 31 December 2015.

The objectives of this study were to:

- determine the positive yield of *Clostridium difficile* in stool samples sent from 576 ICU and 579 HCU at CMJAH from 1 January 2014 until 31 December 2015
- determine the number of stool samples that are positive for *Clostridium difficile* out of the total number of patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015
- determine the number of NAP1 positive samples
- estimate the time from onset of diarrhoea until the time the stool sample was received in the laboratory
- estimate the time from sample received in the laboratory until laboratory diagnosis

- estimate the time from onset of diarrhoea to initiation of treatment
- determine whether the patient was treated empirically or following laboratory diagnosis
- determine whether a patient was started on metronidazole, vancomycin or both
- determine whether APACHE II score differs between a positive and negative *Clostridium difficile* result

A retrospective, descriptive, contextual study design was followed in this study. Following a data request the NHLS provided a list of stool sample that where sent for *Clostridium difficile* testing from 576 ICU and 579 HCU for the period from 1 January 2014 until 31 December 2015. It included a total of 293 stools samples from 192 patients. Of the 293 samples, 10 samples from five patients were excluded as the patients were under the age of 18. Thus a total of 283 samples from 187 patients were included in this study. Following a review of the clinical records of the possible 187 eligible patients only 128 clinical records (179 samples) were available and complete.

Of these samples 38 (13.42%) tested positive for *Clostridium difficile.*

Out of the total number of 3941patients admitted in 576 ICU and 579 HCU from 1 January 2014 until 31 December 2015, 38(0.96%) samples from 26 individual patients (0.66%) sent for *Clostridium difficile* tests were positive.

The NHLS does not test for the NAP1/BI/027 strain and thus we were unable to determine the number of NAP1/BI/027 positive samples.

The time from onset of diarrhoea until a stool sample was received in the laboratory ranged from 0 days (same day) to 61 days. The median time was two days.

The median time taken from sample received at laboratory until reported diagnosis for *Clostridium difficile* PCR was five hours. The median time taken for *Clostridium difficile* toxin test to be performed was 23 hours. The time difference was significant.

The median time form onset of diarrhoea until treatment was initiated was 40 hours.

In this study 24 patients (20%) received empiric treatment. In 77 (64.17%) of patients no treatment was initiated. Of the remaining 43 patients who were started on treatment, 10 (23.33%) were started on oral metronidazole only, 2 (4.65%) were started on intravenous metronidazole only and 28 (65.12%) were started on both oral and intravenous metronidazole.

Only 2 (1.67%) had received vancomycin PO and in both these cases the patients also received both oral and intravenous metronidazole.

The median APACHE II score for CDI negative patients was three with a range of 0-22. The median APACHE II score for CDI positive patients was 22 with a range of 9-37. Thus the APACHE II scores for patients who tested positive for *Clostridium difficile* were significantly higher than those who tested negative ($p < 0.0001$).

5.3 Limitations

This study was contextual and limited to the 576 ICU and 579 HCU population at CMJAH who had stool samples sent to the laboratory *Clostridium difficile* tests during 2014 and 2015. This limits the generalisation of the results. The results may therefore not be applicable to another institution.

The study population was also limited to only patients who had samples sent to the laboratory. Thus the study did not investigate all admissions during the specified time frame or all patients with diarrhoea and as such a true incidence of CDI in 576 ICU and 579 HCU could not be determined.

The study was retrospective in nature and relied on the availability of clinical records. In this study 59 (31.55%) clinical records were excluded as they were not found or the records were incomplete. This is a substantial portion of the study population and had the records been available might have had a significant influence on the results.

Due to the fact that NHLS does not routinely test for the NAP1/BI/027 strain we could not determine the number of samples positive for this particular strain and hence could not meet one of the study objectives.

Lastly we relied on the NHLS to provide a complete data set of all stool samples sent from 576 ICU and 579 HCU during 2014 and 2015. The researcher is unable to verify whether the data set is complete and thus potentially some samples not included in the study.

5.4 Recommendations

5.4.1 Recommendations for clinical practice at 576 ICU and 579 HCU CMJAH

Healthcare workers should be educated about the transmission and spread of CDI as well as effective infection control strategies in order to decrease the burden of disease in our institution Hand hygiene is the cornerstone of preventing the spread of CDI. Washing hands with soap and water is proven to be superior in removing *Clostridium difficile* from hands and it is the recommended method of hand hygiene when CDI is suspected or confirmed (100). Thus washing hands with soap and water should be promoted in the intensive care setting.

When CDI is suspected and early diagnosis is paramount the PCR should be the investigation of choice because of its faster turnaround time at CMJAH .To negate the concerns regarding false positive results the PCR can be used in conjunction with the toxin EIA. Current practice at CMJAH NHLS is to do a EIA GDH prior to the PCR as the EIA GDH has excellent negative predictive value (1, 58).

Current practice in 576 and 579 is to initiate patients with suspected or proven CDI in both oral and intravenous metronidazole. Whether the addition of intravenous metronidazole has any benefit over oral metronidazole has not been investigated yet. Current treatment guidelines from both the ACG and ESCMID are to treat first episodes of mild to moderate CDI with oral metronidazole only (65, 70). Oral metronidazole has been found to be effective in mild to moderate disease resulting in clinical resolution of disease (61). Not only has oral metronidazole been proven effective it is also reduces cost when a single treatment modality is employed instead of multiple treatment routes. We therefore recommend oral metronidazole alone when initiating treatment for CDI.

5.4.2 Recommendations for further research

A national study on the incidence of CDI in South African hospitals is recommended.

The efficacy of oral metronidazole compared to oral and intravenous metronidazole should be evaluated.

5.5 Conclusion

This study investigated the burden of CDI and some aspects of treatment in 576 and 579 during 2014 and 2015. It was found that 13.42% of all samples sent to the laboratory for *Clostridium difficile* test and of the total number of 3941patients admitted in these units during 2014 and 2015 26 individual patients (0.66%) were positive for CDI.

The ideal diagnostic test should have a high sensitivity and specificity, quick turnaround time and be affordable. The GeneXpert ® PCR has a faster turnaround time when compared to the toxin EIA at CMJAH.

We found that 20% of patients received empiric treatment and that oral and intravenous metronidazole is initiated as first line treatment in most cases of suspected or confirmed CDI. Furthermore this study showed that patients who are positive for CDI have significantly higher APACHE II scores than those who do not have CDI.

Institutional knowledge of the burden of disease of CDI as well as current practice can aid in better clinical decision making and improve patient outcome.

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Appendix A

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

12 September 2016
Person No: 1243670
PAG

Dr A Botha
PO Box 506
Wierdapark
0149
South Africa

Dear Dr Botha

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Clostridium difficile in stool samples in an academic hospital's intensive care and high care unit* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix B

R14/49 Dr Amorie Botha

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160668

NAME: (Principal Investigator)	Dr Amorie Botha
DEPARTMENT:	Anaesthesiology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Occurence of Clostrium Difficile in Stool Samples in an Academic Hospital's Intensive Care and High Care Unit
DATE CONSIDERED:	24/06/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	A van den Heever and C Hosking
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	19/09/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year.

Principal Investigator Signature

Date 25/9/2016.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Mr. J. Maepa
Office of the Clinical Director
Tell: (011): 488-3365
Email:Johannes.maepa@gauteng.gov.za
07 July 2016

Dear Dr Amorie Botha

STUDY TITLE: Occurrence of Clostrium Difficile in Stool Samples in an Academic Hospital's Intensive Care and High Care Unit.

Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic Hospital will not anyway incur or inherit costs as result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the HOD and Unit Manager or sister in charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Clinical Director
DATE: 8/07/2016

Approved/not approved

Ms G. Bogoshi
Chief Executive Officer
Date: 11.07.2016

Appendix D

WITS UNIVERSITY

Department of Anaesthesiology
UNIVERSITY OF THE
WITWATERSRAND, JOHANNESBURG
Charlotte Maxeke JHB Academic Hospital
Tel. (011) 488-4344/ Fax. 011 4884343

FACULTY OF HEALTH SCIENCES

Prof Oosthuizen

CMJAH

10/5/2016

To whom it may concern

I hereby grant permission to Dr. Amorie Botha to conduct the study entitled **OCCURRENCE OF CLOSTRIDIUM DIFFICILE IN STOOL SAMPLES IN AN ACADEMIC HOSPITAL'S INTENSIVE CARE AND HIGH CARE UNIT.**

Yours sincerely

Professor Oosthuizen

HOD Anaesthesiology (CMJAH)

10/5/2016

Appendix E

WITS UNIVERSITY

FACULTY OF HEALTH SCIENCES

9 May 2016

To whom it may concern

I hereby grant permission to Dr. Amorie Botha to access the ICU records for the purpose of conducting the study entitled OCCURRENCE OF CLOSTRIDIUM DIFFICILE IN STOOL SAMPLES IN AN ACADEMIC HOSPITAL'S INTENSIVE CARE AND HIGH CARE UNIT.

Yours sincerely

Professor G Richards
Principle Specialist and Director of Critical Care
Charlotte Maxeke Johannesburg Academic Hospital

Telephone: 011 488 3576

Appendix F

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
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31 August 2016

Applicant: Dr Amorie Botha
Institution: University of the Witwatersrand
Department: Anaesthesia – Charlotte Maxeke Johannesburg Academic Hospial
Email: amorie1985@gmail.com
Cell: 082 300 3893

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"Occurrence of Clostrium Difficile in Stool Samples in an Academic Hospital's Intensive Care and High Care Unit"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required will be made available to you to conduct the proposed study as outlined in the submitted protocol.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Department) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research. Once all requirements have been met, please resubmit the completed form to Academic.research@nhls.ac.za for processing by the Corporate Data Warehouse. Any data related queries may be directed to Sue Candy, manager NHLS Corporate Data Warehouse, Tel: (011) 386 6036. Email: sue.candy@nhls.ac.za.

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix G

DIN	GENDER	AGE	WARD_NA	TEST	RESULT	NAP 1	RECEIVED	REPORTED	ADMISSION DX
1	F	36	ICU 576	GXPCD	P	T	29-12-14 10:49	30-12-14 11:13	OBTSRUCTIVE HYDROCEPH POST VP SHUNT, POST FOSSA MASS
1	F	36	ICU 576	TOXIN	P	T	27-12-14 4:12	29-12-14 9:38	
2	F	41	ICU 576	GXPCD	N	PN	19-12-14 13:57	22-12-14 10:59	RVD, POST LAP FOR HYDATID CYSTS
2	F	41	HIGH CARE	TOXIN	N	PN	20-12-14 21:23	23-12-14 14:22	
3	F	37	ICU 576	TOXIN	N	T	16-04-15 4:19	20-04-15 13:55	RVD, PCP, DCMO
3	F	38	ICU 576	GXPCD	N	PN	14-04-15 11:32	14-04-15 15:42	
4	F	39	ICU 576	GXPCD	P	PN	05-11-15 12:58	05-12-15 10:23	MILLIARY TB, ARF, CAP
4	F	39	HIGH CARE	GXPCD	P	PN	14-05-15 9:57	15-05-15 10:52	
5	M	47	ICU 576	TOXIN	N	T	14-06-15 8:44	14-06-15 15:06	CAP, BIBASAL GANGLIA INFARCTS,
6	F	40	ICU 576	GXPCD	N	PN	08-04-15 7:58	08-04-05 14:21	ALCOHOLIC LIVER DISEASE, RVD, ENCEPHALOPATHY
7	M	23	ICU 576	GXPCD	N	PN	08-06-15 9:55	08-06-15 13:13	OESOPHAGECTECTOMY POST CAUSTIC INGESTION
8	M	29	ICU 576	TOXIN	P	T	20-12-14 8:08	20-12-14 15:08	STATUS EPILEPTICUS
9	M	53	ICU 576	TOXIN	N	T	28-06-14 19:29	01-07-14 12:05	RVD, PCP
10	F	39	ICU 576	TOXIN	N	T	09-03-14 0:56	10-03-14 13:58	CEREBRAL MALARIA, AKI,THROMBOCYTOPAENIA
11	F	63	ICU 576	GXPCD	N	PN	22-09-15 13:49	22-09-15 16:10	ILD,CLL, POST HERNIA REPAIR
12	F	37	ICU 576	GXPCD	N	PN	25-11-15 15:27	25-11-15 13:32	
13	F	76	ICU 576	GXPCD	P	PN	02-10-15 13:59	02-10-15 17:32	POST WHIPPLES, HOP MASS
14	F	68	ICU 576	TOXIN	N	T	03-08-15 6:40	03-10-15 9:25	ESRD, LUPUS NEPHRITIS, HEP C, PULM OEDEMA

DIN	DIARRHOEA ONSET TIME	RX STARTED	METRONIDAZOLE- PO	METRONIDAZOLE- IV	VANCO(Y/N)	EMPIRIC(Y/N)	DAYS IN ICU	APACHE II	NO RECORDS
1	26-12-14 17:00	N	N	N	N	N	4	36	
1									
2	17-12-14 22:00	N	N	N	N	N	3	18	
2	17-12-14 22:00						3		
3	13-04-15 5:00	N	N	N	N	N	17	21	
3									
4	08-05-15 21:00	14-05-15 14:00	Y	N	N	N	1	22	
4									
5	14-06-15 22:00	15-06-15 14:00	Y	Y	N	Y	0	31	
6	01-08-15	N	N	N	N	N	4	17	
7	05-08-15 9:00	N	N	N	N	N	6	9	
8	19-12-14 23:00	20-12-14 22:00	Y	Y	N	N	6	25	
9	27-06-14 8:00	N	N	N	N	N	6	17	
10	08-03-14 8:00	N	N	N	N	N	6	30	
11	22-09-15 5:00	22-09-15 10:00	Y	Y	N	Y	4	28	
12									Y
13	09-02-15 7:00	?	?	?	?	?	5	19	
14	07-03-15 9:00	N	N	N	N	N	3	20	