Clostridium difficile **infection at Charlotte Maxeke Johannesburg Academic Hospital**

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine

2016, Johannesburg

DECLARATION:

I, Lee-Anne Godinho, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine 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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ABSTRACT

Background and objectives: In recent years, *Clostridium difficile* infection has become more common and more resistant to therapy resulting in significant in-patient morbidity and mortality. The aim of this study was to describe the characteristics of patients with *Clostridium difficile* infection at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, South Africa.

Methods: This was a retrospective analysis of the records of 154 adult in-patients with confirmed *Clostridium difficile* infection on stool samples during the period 1 January 2013 to 30 June 2014. Patient demographics, clinical presentation, risk factors, HIV status, treatment, severity indicators and outcomes were analysed. Patients were catagorised as having either healthcare-associated or community-acquired *Clostridium difficile* infections. HIV-positive patients were compared with HIV-negative patients and the survivors were compared with the non-survivors.

Results: Overall, 83 (53.8%) patients were female and the median age was 39 years (Interquartile range: 31-52). The most common symptom was watery diarrhoea (92.9%) followed by fever (27.3%). Overall, 145 (97.9%) patients had been exposed to antibiotics in the last 30 days, 54 (35.1%) to immunosuppressives, 48 (31.2%) to gastric acid suppressants and 65 (42.2%) patients had previous hospital admissions. In total 73 (47.4%) patients died. Only two cases of community-acquired *Clostridium difficile* infections were identified. There was no difference in outcomes of HIV-positive patients compared to HIV-negative patients. The non-survivors tended to have a greater number of severity indicators compared to the survivors.

Conclusion: Patients with *Clostridium difficile* infection in this study were younger than expected with a higher mortality than that reported in the Western world. The number of

community-acquired *Clostridium difficile* infections was less than reported in previous studies.

ACKNOWLEGEMENTS

I would like to thank my supervisor Professor Charles Feldman for his guidance and invaluable advice throughout the planning and completion of this study.

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1.0 INTRODUCTION

Clostridium difficile infection is an important cause of in-patient morbidity and mortality. Over recent years the infection has become more common with an increase in resistance to therapy and frequent recurrence. For this reason it has become more important to increase our understanding of this infection in our patient population. There are very little local data available on this subject and the aim of this study was to gain more information regarding the demographic characteristics, common presenting clinical features and identifiable risk factors, treatment and outcomes of patients in Johannesburg, South Africa. The study was a retrospective record review of adult (≥18 years) in-patients at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) with confirmed *Clostridium difficile* infection. Such a study has not been done previously in Johannesburg.

1.1 The organism

Clostridium difficile (CD) is an anaerobic bacillus which stains Gram positive and is known to be spore forming. The organism can asymptomatically colonize the gut or cause a spectrum of disease from mild diarrhoea to extensive pseudomembranous colitis, toxic megacolon and bowel perforation (Rajabally et al. 2013). The CD spores are ingested and germinate in the small intestine where they produce exotoxins which cause mucosal damage and colitis (Gould & McDonald 2008). CD does not directly invade the colon and infection outside of the colon is extremely rare (Leffler & Lamont 2015).

1.2 Risk factors

1.2.1 Drugs

One of the major risk factors for *Clostridium difficile* infection is exposure to antibiotics in the prior 30 days. The most commonly associated antibiotics include the fluoroquinolones,

cephalosporins and clindamycin. These antibiotics cause CD overgrowth and toxin production in the gut by disturbing the normal gut flora. Similarly, prolonged hospital stays increase the risk of CD due to extensive antibiotic use (Bobo, Dubberke & Kollef 2011). One study found that 75% of cases of hospital-acquired CD infections were identified on the 7th day of admission, and only 2.8% and 5.5% were identified on the 3rd and 4th days of admission respectively (Oake, et al. 2010).

In 2010, a study documented that gastric acid suppression increased the risk for nosocomial CD infection (Howell et al. 2010). The risk of CD infection without acid suppressants was found to be 0.3% versus 0.6% in patients on a histamine 2 (H2) receptor antagonist and 0.9% in patients on daily proton pump inhibitors (PPIs). The risk in patients on more frequent than daily PPIs was found to be 1.4%. It is speculated that this is due to vegetative forms of CD that are able to survive longer with neutralization of gastric acid (Freedberg et al. 2010; Linsky et al. 2013).

Immunosuppressive therapies, including chemotherapy and radiation therapy, also increase the risk of CD infection. Drugs that result in intestinal stasis (such as opiates and loperamide) may also increase the risk of CD infection (Bobo, Dubberke & Kollef 2011). Furthermore, The British Journal of Pharmacology published a study which demonstrated that diclofenac is associated with a 35% increased risk of CD-associated disease in non-hospitalized patients (Suissa et al. 2012). This was not found with the use of other non-steroidal anti-inflammatory drugs (NSAIDs).

Another study suggested that depression and/or the use of certain anti-depressants (namely mirtazapine, fluoxetine and nortriptyline) are associated with the development of CD infection (Rogers et al. 2013). In the same study, being widowed or living alone were also suggested risk factors for CD infection. The study suggested that this is because these

patients were more likely to have depressive symptoms which have been shown to cause an increased and prolonged inflammatory response and immune dysregulation. Furthermore, bereavement has been shown to decrease neutrophil production of superoxide radicles and to reduce the functional capacity of natural killer cells. Therefore, the increased risk of CD infection may be related to the physiological changes in depression rather than the use of anti-depressants (Rogers et al. 2013).

1.2.2 Host factors

Obesity has been identified as a risk for CD infection. In a study from 2013, the mean BMI of patients who acquired CD infections was 33.6 versus 28.9 in the control group (Jihad et al. 2013). As mentioned previously, depression is also a host-related risk factor (Rogers et al. 2013). Other host factors include age ≥65 years, multiple comorbidities, peri-partum females, inflammatory bowel disease, chronic kidney disease requiring haemodialysis and human immunodeficiency virus (HIV) infection (Bobo, Dubberke & Kollef. 2011).

1.2.3 HIV infection

The risk of CD infection in HIV-positive individuals is twice that of HIV-negative individuals. This is most true in patients with low CD4 cell counts and Acquired Immunodeficiency Syndrome (AIDS)-defining conditions (Raines & Lopez 2011). This is likely to be caused by the change in their gut microbiome, gut mucosal integrity and abnormal humoral and cell-mediated immunity. However this increased risk can also be attributed to risk factors such as longer hospital admissions and antibiotic use (especially during opportunistic infections) in HIV-seropositive individuals compared to HIV-seronegative individuals. The clinical presentation, severity and outcomes of CD infection appear to be similar in HIV-positive and HIV-negative individuals.

1.3 Healthcare-associated infections (HCAIs) versus community-acquired infections

In general, CD infections are defined as being healthcare-associated infections (HCAIs), nosocomial infections or community-acquired infections. Friedman et al. (2002) described HCAIs as a category of infections that are present at the time of admission or within hours of admission of patients to hospital if the patient had received specific home care (intravenous therapy, wound care or home-based nursing care) or attended a haemodialysis clinic in the days before the infection, if the patient was hospitalised two or more days in the preceding 90 days or if they lived in a nursing home or long-term care facility. The latter group are also known as nursing home-acquired and long-term care-acquired infections. This definition, although originally used to describe bloodstream infections, is widely used in clinical studies regardless of the locus of infection. The Infectious Diseases Society of America defined hospital-acquired (nosocomial) CD infection as occurring >48hrs after admission (therefore not incubating or present at the time of admission) up to 4 weeks (30 days) after hospital discharge (Gupta & Khanna 2014). Community-acquired CD infection is defined as disease onset in a person who has had no overnight stay in a healthcare facility within the last 90 days. This definition does not rule out all contact with the healthcare system (Leffler & Lamont 2015). If symptom onset occurs in the community between 4 to 12 weeks after discharge from a hospital, it is considered 'indeterminate' (Gupta & Khanna 2014).

Since 2005 a marked rise in the incidence of community-acquired CD has been noted. According to Chitnis et al. (2013) an estimated 20 to 25% of CD infections are community-acquired with an incidence of 20 to 50 cases per 100 000 population in the United States, Sweden and England. A South African study in Cape Town identified 59 patients with CD-associated diarrhoea of which 19 (32.2%) were community-acquired (Rajabally et al. 2013).

Community-acquired CD infection tends to affect younger patients with fewer comorbidities (Leffler & Lamont 2015). An epidemiologic study undertaken in the UK showed that almost all the cases of community-acquired CD infection were <65 years old (Gupta & Khanna 2014). Despite a lower morbidity and mortality for community-acquired CD infection, up to 40% of patients will require hospitalization (Leffler & Lamont 2015). The presence of severe infection as well as increasing age, increased number of comorbidities and failure to respond to therapy predict the need for hospitalization (Gupta & Khanna 2014).

The study by Chitnis et al. (2013) evaluated out-patient healthcare exposure in patients with community-acquired CD infection in the prior 12 weeks. The healthcare exposure was separated into 3 categories namely: no exposure, low level exposure (out-patient doctor or dentist visit) or high level exposure (surgery, emergency visits, in-patient care without overnight stay or health care personnel). It was found that only 18% of patients had no healthcare exposure. Identifiable risk factors included exposure to antibiotics, gastric acid suppression or household members with active CD infection as person-to-person (faeco-oral) transmission is the primary route of transmission rather than zoonotic transmission or food contamination (Chitnis et al. 2013; Gupta & Khanna 2014).

1.4 Severity of disease

According to the guideline for diagnosis, treatment and prevention of CD infections (Surawicz et al.), the infection is considered to be mild to moderate with the presence of diarrhoea but without any features of severity. “Severe disease” is considered to be present with an albumin of <30g/L plus one of either: WCC $\geq 15\times10^9$/L or abdominal tenderness. The disease is considered “severe and complicated” when the patient is admitted to an intensive care unit attributable to CD infection, is hypotensive with or without the use of inotropes or has any of the following features including: fever ≥38.5°C, ileus or significant abdominal

distension (pseudomembranous colitis on endoscopy or toxic megacolon), altered mental state, WCC $\geq 35\times10^9$/L or $<2\times10^9$/L, serum lactate >2.2mmol/L or evidence of end organ failure (e.g. requires mechanical ventilation or has renal failure) (Surawicz et al. 2013).

1.5 Morbidity and mortality

Mitchell and Gardner (2012) reviewed literature from 2005 to 2011 to find that 15 studies indicated a mortality of 15% or greater in CD infection. Another study reviewed 136,877 admissions to Ottawa Hospital in Canada and found that hospital-acquired CD infection was associated with an increased risk of in-hospital death regardless of baseline mortality risk. Hospital-acquired CD infection increased the absolute risk of death by approximately 10% (Oake et al. 2010).

In the last few years an increase in the incidence and severity of CD infection has been noted, some of which is thought to be related to the emergence of a hypervirulent strain of CD (Gould & McDonald 2008). This strain has been termed restriction enzyme analysis type B1, North American Pulsed-Field Type 1 (NAP1) and PCR ribotype 027. It produces 16 times the concentration of toxin A, 23 times the concentration of toxin B, as well as an additional binary toxin rendering it more virulent. The role of this binary toxin has not yet been established; however it is associated with more severe diarrhoea. This strain also demonstrates high levels of resistance to fluoroquinolones.

Variables that have been associated with increased mortality include: albumin $\leq$ 24.5g/L, white cell count (WCC) $>12\times10^9$/L, C-Reactive Protein (CRP) >228mg/L and respiratory rate >17 breaths per minute (Butt et al. 2013). Another study found age >70 years, WCC $>35\times10^9$/L or $<4\times10^9$/L and cardiorespiratory failure to be independent predictors of mortality (Bobo, Dubberke & Kollef 2011).

1.6 Treatment

Metronidazole and vancomycin are the most commonly used antibiotics for the treatment of CD infection. Treatment for CD usually involves stopping the offending antibiotics, if possible, and initiating therapy with oral metronidazole (500mg 8 hourly) for 10-14 days (Collini et al. 2013). In cases of a high clinical suspicion for CD infection, treatment should be initiated empirically prior to stool testing. Oral metronidazole at this dose is recommended for mild to moderate disease. However, in patients intolerant/allergic to metronidazole or in pregnant/breastfeeding females, vancomycin should be used instead (Surawicz et al. 2013).

Treatment response is when the stool frequency decreases or stool consistency improves and parameters of disease severity improve without the development of new features of severity (Debast, Bauer & Kuijper 2013). Poor response to metronidazole within 5-7 days should prompt a change to vancomycin (Surawicz et al. 2013).

Severe cases are managed with oral vancomycin (125mg) four times daily (6 hourly) for 10-14 days as vancomycin has been shown to be superior to metronidazole in the treatment of severe disease. When oral antibiotics cannot reach a segment of colon, for example, with a Hartman pouch, ileostomy or colon diversion, vancomycin via enema should be added to treatment. Patients with “severe and complicated” CD infection should be managed supportively and treated with vancomycin 125mg 6 hourly plus intravenous metronidazole (500mg 8 hourly). In the case of ileus or toxic megacolon, 500mg vancomycin should be given orally 6 hourly as well as 500mg (in 500ml fluid) per rectum plus intravenous metronidazole (500mg 8 hourly). Complicated CD infection warrants urgent CT abdomen and pelvis and surgical consultation (Surawicz et al. 2013). Probiotics incorporating lactobacilli and bifidobacteria have not been shown to be effective in prevention of antibiotic-associated diarrhoea or in CD-associated diarrhoea (Allen et al. 2013).

1.7 Recurrence

Recurrence is defined by the Infectious Disease Society of America/Society for Healthcare Epidemiology of America (IDSA/SHEA) as the presence of diarrhoea with a positive *Clostridium difficile* stool assay within 2–8 weeks from the initial episode (Cohen et al. 2010). Risk factors for recurrence of CD infection include age ≥65 years, PPI use while on treatment for CD, increased hospitalization and prolonged hospital stay, as well as severe underlying disease such as chronic renal disease, inflammatory bowel disease and immuno-compromise (Kelly 2012). In these patients repeated antibiotics are being used; however infusion of donor faeces has shown efficacy in treating these patients. Infusion antibodies against CD toxins have to be investigated as to whether they are an effective treatment strategy (Van Nood et al. 2013). Fidaxomicin is an effective treatment that can be used in severe infections as its cure rate has been shown to be equivalent to that of vancomycin. The risk of recurrence of disease has been shown to be 15% with fidaxomicin compared to 25% with vancomycin. However this reduced rate of recurrence was not seen with the NAP1 PCR ribotype 027 strain (Leffler & Lamont 2015).

1.8 South African perspective

There are only five *Clostridium difficile* studies that have been undertaken in South Africa. Two of these were in paediatric patients and will not be discussed here. A study was undertaken in Pretoria by Storm et al. (2012) where stool samples were analysed for the presence of toxin A, B and binary toxin genes. It was found that conventional polymerase chain reaction (PCR) testing is useful for detecting all of the toxin genes of CD in stool specimens. Toxin A producing strains of CD were found to be the most prevalent in Pretoria but may have the potential to acquire binary toxins which may cause severe disease. Another study was undertaken in the Vhembe district of Limpopo by Samie et al. (2008). In this

study, PCR testing was used to determine the prevalence of toxigenic CD. Out of 322 stools collected, 7.1% contained toxigenic CD and 11.4% were associated with diarrhoea. Toxigenic CD was found to be more common in individuals older than 50 years (20%), followed by the 30-39 year age category (19%). Co-infections were common, in particular, with *Enterocytozoon bieneusi*, *Cryptosporidium parvum* and *Escherichia coli*.

A 2013 study done at Groote Schuur Hospital in Cape Town by Rajabally et al. aimed at identifying risk factors and patient outcomes. In that study 58% of patients with CD infection were found to be female, 66% had prior antibiotic exposure, 46% had been hospitalised in the last 3 months and 24% had concomitant inflammatory bowel disease. The study found that hospital-acquired CD infection was much less common in South Africa (0.87 cases per 1000 hospitalisations) as compared to the first world (7.4 cases per 1000 hospitalizations). It was also reported that although antibiotic use was a major risk factor for nosocomial CD infection, most cases of community-acquired CD infection were not associated with antibiotic exposure. That study paves the way for further investigation as it begs the question as to what else is unique regarding this infection in the South African setting?

Similar data needed to be reviewed in Johannesburg with regards to understanding patient risk factors, presenting features, treatment and outcomes. There is also a need for comparison of HIV-positive versus HIV-negative patients and HCAIs versus community-acquired infections. Another aspect to consider is the comparison of survivors and non-survivors in order to better understand factors affecting the outcome of the condition.

2.0 METHODS

2.1 Study population and data collection methods

The study was a single-center, retrospective analysis of in-patients with confirmed *Clostridium difficile* infection. The study was conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a tertiary academic hospital in Gauteng Province, South Africa. The study included adult patients (≥18 years) from the infectious diseases wards (497 and 587), gastroenterology ward (595) and the intensive care unit (ICU; 576) during the period of 1 January 2013 to 30 June 2014 with confirmed *Clostridium difficile* infection. A list of all the patients whose stool samples tested *Clostridium difficile* positive at Charlotte Maxeke Hospital during this 18 month time period was obtained from the National Health Laboratory Service (NHLS) database and the selected wards were filtered out. Data was collected from hospital records and/or ICU charts obtained from the hospital records department. Data was extracted from these documents using a data capturing sheet (see Appendix A). Patients with incomplete and missing files were excluded from the study. Ethics approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (see Appendix A).

2.2 Patient variables collected

Patient demographics, including age and gender, were recorded. In particular, it was noted whether patients were older than 65 years of age or not, as this is a known risk factor for *Clostridium difficile* infection. Patients younger than 18 years of age were excluded from the study. The presenting signs and symptoms including watery/bloody diarrhoea, fever (>38°C), abdominal pain, nausea, vomiting, loss of appetite, peritonism and ileus were recorded. Exposure to antibiotics within the last 30 days and the types of antibiotics were recorded. Other risk factors that were recorded included the use of: gastric acid suppressants

(antacids/H2 receptor antagonists/PPIs), immunosuppressive drugs (oral or intravenous corticosteroids/other), diclofenac, anti-depressants (fluoxetine/mirtazapine/nortriptyline) or drugs that cause intestinal stasis e.g. loperamide or opioids (morphine/pethidine/codeine/tramadol).

HIV status, CD4 cell counts, HIV viral loads and the use of anti-retroviral or tuberculosis (TB) therapy was recorded. The duration of hospital stay and the number of previous hospital admissions were recorded. It was noted whether previous hospital admissions were <2 or ≥2 days duration and whether they occurred more than or less than 90 days ago. It was also noted whether the current CD infection symptoms began prior to or after 48 hours of hospital admission. This was used to categorise admissions into either health-care associated infections (HCAIs) or community-acquired infections. HCAIs were defined as being present at the time of admission or within hours of admission if the patient had received specific home care (intravenous therapy, wound care or home-based nursing care) or attended a haemodialysis clinic in the days before the infection, if the patient had been hospitalised for two or more days in the preceding 90 days or if they lived in a nursing home or long-term care facility (Friedman et al. 2002). Hospital-acquired (nosocomial) CD infections were defined as those that occurred during a hospital admission beginning >48 hours after admission and up to 4 weeks (30 days) after hospital discharge. (Gupta & Khanna 2014). For the purpose of the current study, hospital-acquired infections were included together with HCAIs. Community-acquired CD infections were defined as disease onset in a person who had no overnight stay in a healthcare facility within the last 90 days (Leffler & Lamont 2015). This definition does not rule out all contact with the healthcare system or acquisition in a healthcare facility as patients may have attended out-patient clinics or general practitioners.

The presence of certain known severity and mortality indicators were noted including: albumin ≤24.5g/L, WCC >12×10^9/L, respiratory rate >17 breaths per minute, CRP >228mg/L, temperature >38.5°C and raised serum creatinine (>90 µmol/L) (Butt et al. 2013). Other common laboratory parameters were also noted, including: white cell count, haemoglobin, platelet count, sodium, creatinine and albumin. The type of treatment administered and duration of treatment was noted from each file. The overall outcome of each patient was recorded, namely: death (non-survivors), discharge (survivors) or unknown outcome (this included transfers to other hospitals or patients who signed the refusal of hospital treatment (RHT) form).

2.3 Sample size

Data was collected for 154 patients. Sample size calculations were based on the key research questions, namely the between-group tests, which require the application of the chi-square test to determine the association between two categorical variables. Based on the 5% significance level and 80% power, Table 2.1 shows the sample sizes required for the detection of small, medium and large effect sizes. A sample size of 154 was thus adequate to detect a medium effect size as most of the comparisons in this study required 2x2 table sizes.

Table 2.1: Sample sizes required to detect small, medium and large effect sizes in a chi-square test at the 5% significance level and at 80% power

Table size (number of categories in each variable)	Sample size required		
	Large effect size	Medium effect size	Small effect size
2 x 2	31	87	785
3 x 2	39	108	964
4 x 2	44	122	1091
5 x 2	48	133	1194

Sample size calculations were carried out in G*Power (Buchner 2007)

2.4 Data analysis

Categorical variables were summarised in terms of frequency and percentage and were tabulated. Continuous variables were summarised in terms of mean and standard deviation or median and interquartile range after the inspection of histograms.

HIV-positive and HIV negative patients were compared in terms of demographics, presenting symptoms/signs, risk factors, severity indicators and outcomes. The survivors and non-survivors were compared in terms of demographics, risk factors, duration of hospital stay, previous admissions, treatment type, treatment duration and severity/mortality indicators. The chi-square test was used to assess these relationships for categorical variables. Fisher's exact test was used for 2x2 tables and where the requirements of the chi-square test could not be met. The strength of the associations was measured by Cramer's V and the phi coefficient. The scale of interpretation is given in Table 2.2.

Table 2.2: Strength of association according to Cramer's V or phi coefficient

Cramer's V or phi coefficient	**Strength of association**
0.5 and above	Strong association
0.3-0.49	Moderate association
0.1-0.29	Weak association
Below 0.1	Little or no association

For duration of hospital stay, the comparisons were carried out using the Wilcoxon rank sum test. Data analysis was carried out using SAS (version 9.4 for Windows) with the assistance of DMSA (Data Management and Statistical Analysis). The 5% significance level was used.

3.0 RESULTS

3.1 Demographics

Overall 154 patients were included in the study of whom 83/154 (53.8%) were female and 71/154 (46.1%) were male. The median age was 39 years (interquartile range: 31-52). The youngest patient was 19 years old, the oldest patient was 92 years old and 20/154 (13%) patients were older than 65 years. In total, 105/154 (68.2%) patients were HIV-positive, 39 (25.3%) were HIV-negative and 10 (6.5%) were of unknown HIV status.

3.2 Clinical presentation: signs and symptoms

The presenting signs and symptoms in patients who were *Clostridium difficile* positive are indicated in Table 3.1. The most common symptom was watery diarrhoea (92.9%) followed by fever (27.3%). A single case each of toxic megacolon and pseudomembranous colitis was found.

Table 3.1: Presenting signs and symptoms in patients with *Clostridium difficile* infection

Signs and symptoms	**No of patients out of 154 (Percentage)**
Watery diarrhoea	143 (92.9%)
Fever (>38°C)	42 (27.3%)
Abdominal pain	25 (16.2%)
Nausea and/or vomiting	18 (11.7%)
Loss of appetite	16 (10.4%)
Bloody diarrhoea	9 (5.8%)
Ileus	3 (1.9%)
Peritonism	1 (0.6%)
Toxic megacolon	1 (0.6%)
Pseudomembranous colitis	1 (0.6%)

3.3 Medication as risk factors

With regards to medications as risk factors for acquiring *Clostridium difficile* infection, a number of the patients had several risk factors, as shown in Table 3.2. Overall, 145/148 (97.9%) patients had been exposed to antibiotics within the last 30 days, 48/154 (31.2%) had been exposed to gastric acid suppressants and 35/154 (22.7%) had been exposed to opioids. In total, 54/154 (35.1%) patients had been given immunosuppressants.

Table 3.2: Medications as risk factors for acquiring *Clostridium difficile* infection

Type of medication patients were exposed to	**n/N(Percentage)**
Exposure to antibiotics in prior 30 days (exposure to antibiotics unknown in 6 patients)	145/148 (97.9%)
Immunosuppressives	54/154 (35.1%)
Gastric acid suppression (H2 receptor antagonist/PPI/antacid)	48/154 (31.2%)
Opioids	35/154 (22.7%)
Loperamide	1/154 (0.6%)
Antidepressants (mirtazapine/nortryptiline/fluoxetine)	0/154 (0%)
Diclofenac	0/154 (0%)

n = number of patients who were exposed to specific medication, N = total number of patients

The types of antibiotics, gastric acid suppressants, opioids and immunosuppressives that patients had received are shown in Table 3.3. In addition in the table it is indicated whether the patients were on TB treatment and/or anti-retroviral therapy. The most common antibiotic that had been given was amoxicillin-clavulanic acid (67/145: 46.2%), followed by piperacillin-tazobactam (39/145: 26.9%) and ertapenem (35/145: 24.1%). The patients were exposed to a median of 2 antibiotics. Of the 48 patients exposed to gastric acid suppressants, 26 (54.2%) were on an oral PPI and 24 (50%) were on an intravenous PPI. Of the 54 patients on immunosuppressants, 39 (72.2%) were on an intravenous corticosteroid. A total of 52.6% of patients were on TB therapy and of the 105 HIV-positive patients, 46.7% were on anti-retroviral therapy.

Table 3.3: Types of medications patients with *Clostridium difficile* infection were exposed to

Types of medications	**n/N (Percentage)**
Types of antibiotics:	
Amoxicillin-clavulanic acid	67/145 (46.2%)
Piperacillin-tazobactam	39 /145 (26.9%)
Ertapenem	35 /145 (24.1%)
3rd generation cephalosporin	34 /145 (23.4%)
Fluoroquinolone	19 /145 (13.1%)
Meropenem	11 /145 (7.6%)
2nd generation cephalosporin	10 /145 (6.9%)
4th generation cephalosporin	10 /145 (3.9%)
Vancomycin	8 /145 (5.5%)
Clindamycin	5 /145 (3.4%)
Cloxacillin	3 /145 (2.1%)
Imipenem	2 /145 (1.4%)
Exposed to unknown antibiotic	7 /145 (4.8%)
Exposed to other antibiotic	39 /145 (26.9%)
Types of immunosuppressives:	
IV corticosteroid	39 /54 (72.2%)
Oral corticosteroid	8 /54 (14.8%)
Oral and IV corticosteroid	6 /54 (11.1%)
Other immunosuppressive	1 /54 (1.9%)
Types of gastric acid suppressants:	
Oral PPI	26/48 (54.2%)
IV PPI	24/48 (50.0%)
Histamine 2 receptor antagonists	1/48 (2.1%)
Antacid	1/48 (2.1%)
Types of opioids:	
Tramadol	18 /35 (51.4%)
Morphine	14 /35 (40.0%)
Codeine	4 /35 (11.4%)
Pethidine	1 /35 (2.9%)
Patients on TB treatment	81 /154 (52.6%)
HIV-positive patients on ARVs	49 /105 (46.7%)

IV = intravenous, PPI = Proton Pump Inhibitor, TB = tuberculosis, ARVs = anti-retrovirals, n = number of cases receiving the specific drug, N = number of patients exposed to that class of drug.

3.4 Previous hospital contact

The number of previous admissions and the number of days admitted to the ward or ICU is documented in Table 3.4. Of the 154 patients, 65 (42.2%) had previously been admitted

within the last 90 days and 6 (9.2%) had more than one previous admission. The median number of days admitted was 17 days (interquartile range: 10-28.5). Overall, 47/154 (30.5%) of patients had been in ICU and the median number of days in ICU was 10 days (interquartile range: 5-14). Only 2/154 (1.3%) of infections were identified as community-acquired and 151/154 (98.1%) of cases were healthcare-associated infections. One patient could not be identified as having either a community-acquired or healthcare-associated infection.

Table 3.4: Previous hospital admissions and number of days patients were admitted to the ward/ICU

Variable	**n/N (Percentage)**
Previously admitted	65/154 (42.2%)
Previous admissions unknown	14/154 (9.1%)
Not previously admitted	75/154 (48.7%)
Of those patient who had been previously admitted (n=65):	
≤90 days ago	63/65 (96.9%)
>90 days ago	2/65 (3.1%)
<2 days duration	2/65 (3.1%)
≥2 days duration	37/65 (56.9%)
Unknown duration of previous admission	26/65 (40.0%)
More than one previous admission	6/65 (9.2%)
Median (IQR) days admitted	17 (10-28.5)
Duration of current admission unknown	3/154 (1.9%)
Minimum to maximum number of days admitted	1-144
No. of patients in ICU	47/154 (30.5%)
Median (IQR) duration of admission in ICU	10 (5.0-14)
Minimum to maximum no of days admitted in ICU	1-48
No. of healthcare-associated infections	151/154 (98.1%)
No. of community-acquired infections	2/154 (1.3%)
Indeterminate if healthcare-associated or community-acquired	1/154 (0.6%)
Untreated patients that died	12/22 (54.1%)
Treated patients that died	59/129 (45.7%)

IQR = interquartile range, ICU = intensive care unit, n = number of patients with that specific variable, N = total number of patients.

3.5 Laboratory parameters

The values of common laboratory parameters are indicated in Table 3.5. The median CD4 cell count for HIV-positive patients in the study was 59×10^6/L (interquartile range: 13.5 – 131.5)

Table 3.5: Laboratory parameters in patients with *Clostridium difficile* infection

Lab parameter	**Mean (standard deviation)/Median (Interquartile range)**
Median WCC ($\times10^9$/L)	8.26 (5.05-13.49)
Mean haemoglobin (g/dL)	9.7 (2.2)
Median platelet count ($\times10^9$/L)	234 (133-364)
Mean sodium (mmol/L)	139.7 (75)
Median creatinine (µmol/L)	73 (50-168.25)
Median albumin (g/L)	22 (19.5-27.0)
Median CRP (mg/L)	113 (66-189)
Median CD4 ($\times10^6$/L)	59 (13.5-131.5)
Median HIV viral load	142195 (343.5-429437)

3.6 Severity indicators

The frequency of each severity/mortality indicator is documented in table 3.6. Of the 154 patients, 91 (59.1%) had an albumin of ≤24.5g/L. This was the most common severity indicator present in the study population.

Table 3.6: Severity/mortality indicators in patients with *Clostridium difficile* infection

Severity indicator (no of cases in which the laboratory parameter was known)	**No of patients (Percentage)**
Albumin ≤24.5g/L (147)	91 (59.1%)
Respiratory rate >17 breaths per minute (154)	77 (50.0%)
Raised creatinine: >90µmol/L (154)	56 (36.4%)
WCC $>12\times10^9$/L (153)	47 (30.5%)
Temperature >38.5°C (154)	37 (24.0%)
CRP >228mg/L (141)	15 (9.7%)

3.7 Treatment

Twenty-two (14.3%) patients did not appear to have been treated for *Clostridium difficile.* The medical records of these patients did not indicate any evidence of them having been treated for the infection. Eighty-five (66.4%) of the treated patients were treated with oral metronidazole 400mg eight hourly and 37 patients (28%), were treated with oral vancomycin. Eighty-five (66.4%) of patients were treated for 10-14 days (Table 3.7).

Table 3.7: Type of treatment and duration of treatment administered for *Clostridium difficile* infection

Type/duration of treatment	**n/N (Percentage)**
Treated patients	128/154 (83.1%)
Patients not treated	22/154 (14.3%)
Treatment unknown	4/154 (2.6%)
Oral metronidazole 400mg 8hrly	85/128 (66.4%)
Oral and IV metronidazole	34/128 (26.6%)
Oral metronidazole 800mg 8hrly	5/128 (3.9%)
IV metronidazole (500mg 8hrly)	4/128 (3.1%)
Oral vancomycin 125mg 6hrly	18/128 (14.1%)
Oral vancomycin 250mg 6hrly	14/128 (10.9%)
Other oral vancomycin dose	5/128 (3.9%)
Treatment given for 10-14 days	85/128 (66.4%)
Treatment given for <10 days	40/128 (31.3%)
Treatment given for unknown duration	3/128 (2.3%)
Treated until death	49/154 (31.8%)

IV = intravenous, Other oral vancomycin dose = 1g daily, 500mg 6 hourly (2 patients), 500mg 8 hourly and 125mg 12 hourly, n = number of patients treated with specific medication/dose, N = total number of treated patients.

3.8 Outcomes

Overall, 73/154 (47.4%) of patients died and 67/154 (43.5%) survived and were discharged. The remaining 14 (9%) patients were either transferred to other hospitals or signed the refusal of hospital treatment (RHT) form and their outcomes are unknown. These findings are demonstrated in Table 3.8.

Table 3.8: Outcomes of patients with *Clostridium difficile* infection

Outcome	**No of patients out of 154 (Percentage)**
Death	73 (47.4)
Discharged	67 (43.5%)
Transfer	10 (6.5%)
Unknown	3 (1.9%)
Signed refusal of hospital treatment form	1 (0.6%)

3.9 Comparison of survivors and non-survivors of *Clostridium difficile* infection

When the survivors and non-survivors were compared, no significant differences were found with regards to demographics and clinical presentation (signs/symptoms). This is recorded in Table 3.9.

Table 3.9: Comparison of survivors and non-survivors of *Clostridium difficile* infection with regards to demographics and clinical presentation (signs and symptoms)

Variable	**Category**	**Overall n (%)**	**Died n (%)**	**Survived n (%)**	**p-value**
N		**154**	**73**	**67**	
Gender	Female	83 (53.9)	37 (50.7)	36 (53.7)	0.74
	Male	71 (46.1)	36 (49.3)	31 (46.3)	
Age	<65 years	134 (87.0)	62 (84.9)	60 (89.6)	0.46
	≥65 years	20 (13.0)	11 (15.1)	7 (10.4)	
Clinical presentation (symptoms and signs)	Watery diarrhoea	143 (92.9)	70 (95.9)	59 (88.1)	0.12
	Bloody diarrhoea	9 (5.8)	2 (2.7)	7 (10.4)	0.09
	Fever (>38°C)	42 (27.3)	24 (32.9)	16 (23.9)	0.27
	Abdominal pain	25 (16.2)	9 (12.3)	12 (17.9)	0.48
	Nausea and/or vomiting	18 (11.7)	8 (11.0)	8 (11.9)	>0.99
	Loss of appetite	16 (10.4)	8 (11.0)	4 (6.0)	0.37
	Peritonism	1 (0.6)	1 (1.4)	0 (0.0)	>0.99
	Ileus	3 (1.9)	3 (4.1)	0 (0.0)	0.25
	Other	2 (1.3)	2 (2.7)	0 (0.0)	

When comparing the survivors and non-survivors of CD infection, there was no difference in terms of exposure to antibiotics, gastric acid suppressants, opioids, immunosuppresants, antidepressants, diclofenac or loperamide. This is demonstrated in Table 3.10.

Table 3.10: Comparison of survivors and non-survivors of *Clostridium difficile* infection in terms of medications as risk factors

Risk factor	**Category**	**Overall n (%)**	**Died n (%)**	**Survived n (%)**	**p-value**
N		**154**	**73**	**67**	
Antibiotics in prior 30 days	Unknown	6 (3.9)	1 (1.4)	3 (4.5)	
	No	3 (1.9)	0 (0.0)	3 (4.5)	0.1
	Yes	145 (94.2)	72 (98.6)	61 (91.0)	
Gastric acid suppression	No	106 (68.8)	50 (68.5)	46 (68.7)	>0.99
	Yes	48 (31.2)	23 (31.5)	21 (31.3)	
Opioids	No	119 (77.3)	58 (79.5)	49 (73.1)	0.43
	Yes	35 (22.7)	15 (20.5)	18 (26.9)	
Immunosuppressives	No	100 (64.9)	43 (58.9)	46 (68.7)	0.29
	Yes	54 (35.1)	30 (41.1)	21 (31.3)	
Anti-depressants	No	154 (100.0)	73 (100.0)	37 (100.0)	
	Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Loperamide	No	153 (99.4)	73 (100.0)	66 (98.5)	0.48
	Yes	1 (0.6)	0 (0.0)	1 (1.5)	
Diclofenac	No	154 (100.0)	73 (100.0)	67 (100.0)	
	Yes	0 (0.0)	0 (0.0)	0 (0.0)	

IV = intravenous

The survivors and non-survivors were also compared in terms of each specific type of medication; however no statistically significant differences could be found. This is demonstrated in table 3.11.

Table 3.11: Comparison of survivors and non-survivors of *Clostridium difficile* infection in terms of exposure to specific medications

Risk factor	**Category**	**Died n (%)**	**Survived n (%)**	**p-value**
N		**73**	**67**	
Antibiotic	Unknown	5 (6.9)	3 (4.9)	
Amoxicillin-clavulanic acid	No	29 (40.3)	32 (52.5)	0.21
	Yes	38 (52.8)	26 (42.6)	
Cloxacillin	No	65 (90.3)	57 (93.4)	>0.99
	Yes	2 (2.8)	1 (1.6)	
Clindamycin	No	66 (91.7)	54 (88.5)	0.18
	Yes	1 (1.4)	4 (6.6)	
Fluoroquinolone	No	59 (81.9)	50 (82.0)	0.79
	Yes	5 (6.9)	3 (4.9)	
Piperacillin-tazobactam	No	44 (61.1)	45 (73.8)	0.17
	Yes	23 (31.9)	13 (21.3)	
Ertapenem	No	51 (70.8)	43 (70.5)	0.84
	Yes	16 (22.2)	15 (24.6)	
Imipenem	No	65 (90.3)	58 (95.1)	0.50
	Yes	2 (2.8)	0 (0.0)	
Meropenem	No	60 (80.3)	54 (88.5)	0.54
	Yes	7 (9.7))	4 (6.6)	
Vancomycin	No	63 (87.5)	54 (88.5)	>0.99
	Yes	4 (5.6)	4 (6.6)	
2nd generation cephalosporin	No	61 (84.7)	54 (88.5)	0.75
	Yes	6 (8.3)	4 (6.6	
3rd generation cephalosporin	No	46 (63.9)	46 (75.4)	0.22
	Yes	21 (29.2)	12 (19.7)	
4th generation cephalosporin	No	62 (86.1)	54 (88.5)	>0.99
	Yes	5 (6.9)	4 (6.6)	
Gastric acid suppressant				
Antacid	No	22 (95.7)	20 (95.2)	>0.99
	Yes	1 (4.3)	0 (0.0)	
Histamine 2 receptor antagonist	No	22 (95.7)	21 (100.0)	>0.99
	Yes	1 (4.3)	0 (0.0)	
Oral Proton pump inhibitor	No	12 (52.2)	6 (28.6)	0.14
	Yes	11 (47.8)	15 (71.4)	
Intravenous (IV) Proton pump inhibitor	No	11 (47.8)	13 (61.9)	0.38
	Yes	12 (52.2)	8 (38.1)	
Opioids				
Morphine	No	7 (46.7)	13 (72.2)	Group size too small for between group comparisons
	Yes	8 (53.3)	5 (27.8)	
Codeine	No	13 (86.7)	16 (88.9)	
	Yes	2 (13.3)	2 (11.1)	
Pethidine	No	15 (100.0)	17 (94.4)	
	Yes	0 (0.0)	1 (5.6)	
Tramadol	No	9 (60.0)	7 (38.9)	
	Yes	6 (40.0)	11 (31.1)	
Immunosuppressives				
Oral corticosteroid	No	27 (90.0)	17 (81.0)	0.43
	Yes	3 (10.0)	4 (19.0)	
Intravenous corticosteroid	No	6 (20.0)	7 (33.3)	0.34
	Yes	24 (80.0)	14 (66.6)	
Oral and intravenous corticosteroid	No	28 (93.3)	18 (85.7)	0.64
	Yes	2 (6.7)	3 (14.3)	

Previous hospital admissions, duration of current hospital admission and whether or not patients were admitted to the intensive care unit (ICU) or not had no impact on survival of patients with *Clostridium difficile* infection. This is indicated in Table 3.12.

Table 3.12: Comparison of survivors and non-survivors of *Clostridium difficile* infection in terms of previous hospital admissions, duration of current hospital admission and whether they were admitted in the intensive care unit (ICU) or not

Risk factor	**Category**	**Died n (%)**	**Survived n (%)**	**p-value**
N		**73**	**67**	
Previous hospital admissions	Unknown	8 (11.0)	4 (6.0)	
	No	39 (53.4)	33 (49.3)	0.48
	Yes	26 (35.6)	30 (44.8)	
Number of previous hospital admissions	1	24 (92.3)	26 (86.7)	0.67
	2	2 (7.7)	4 (13.3)	
Number of days currently admitted to hospital	Median	15	18.5	0.13
	Interquartile range	9-28	12-31.5	
Admitted to ICU	No	47 (64.4)	52 (77.6)	0.10
	Yes	26 (35.6)	15 (22.4)	

ICU = intensive care unit

With regards to the comparison of treatment in the survivors and non-survivors, 16.4% of the survivors were not treated for the infection compared to 10.4% of the non-survivors (p = 0.33). A greater proportion of the survivors (77.6%) were treated with oral metronidazole 400mg 8 hourly as compared to the non-survivors (50.8%) (p = 0.0037; phi coefficient = 0.28). Furthermore, 23/73 (39%) of the patients who died were treated with oral and intravenous metronidazole as compared to only 10/67 (17.2%) of the patients who survived (p = 0.013; phi coefficient = 0.24). Overall, 98% of the survivors had been treated for 10 or more days as compared to only 39% of those who died (p <0.0001; phi coefficient = 0.64). This is demonstrated in table 3.13.

Table 3.13: Comparison of treatment type and duration in survivors and non-survivors of *Clostridium difficile* infection

	Category	**Died n (%)**	**Survived n (%)**	**p-value**
N		**73**	**67**	
Treatment (n = 128)	Unknown	2 (2.7)	2 (3.0)	
	No	12 (16.4)	7 (10.4)	0.33
	Yes	59 (80.8)	58 (86.6)	
Type of treatment	Oral metronidazole 400mg 8 hrly	30 (50.8)	45 (77.6)	0.0037 (phi=0.28)
	Oral metronidazole 800mg 8hrly	3 (5.1)	2 (3.4)	>0.99
	IV metronidazole	3 (5.1%)	1 (1.7)	0.62
	Oral and IV metronidazole	23 (39)	10 (17.2)	0.013 (phi=0.24)
	Oral vancomycin 125mg 8hrly	8 (13.6)	10 (17.2)	0.62
	Oral vancomycin 250mg 8 hrly	8 (13.6)	6 (10.3)	0.78
	Other oral vancomycin dose	2 (3.4)	2 (3.4)	>0.99
Treatment duration	<10 days	34 (60.7)	1 (1.7)	<0.0001 (phi=0.64)
	10-14 days	22 (39.3)	57 (98.3)	

IV = intravenous, Other oral vancomycin dose = 1g daily, 500mg 6 hourly (2 patients), 500mg 8 hourly and 125mg 12 hourly.

When comparing the severity indicators in the survivors and non-survivors, the patients who died had a greater number of severity indicators ($p = 0.0006$; Cramer's $V = 0.39$). Overall, 71.2% of the non-survivors had a respiratory rate above 17 breaths per minute compared to only 28.4% of the survivors ($p < 0.0001$; phi coefficient = 0.43). Raised serum creatinine tended to be more common in non-survivors (33%) compared to survivors (20%) however this finding did not reach statistical significance ($p = 0.08$). This is indicated in Table 3.14.

Table 3.14: Comparison of survivors and non-survivors of *Clostridium difficile* infection in terms of type and number of severity indicators

Severity indicator	**Category**	**Died n (%)**	**Survived n (%)**	**p-value**
N		**73**	**67**	
Number of severity indicators present	Unknown	2 (2.7)	14 (20.9)	
	0	1 (1.4)	12 (17.9)	0.0006 (V=0.39)
	1	12 (16.4)	14 (20.9)	
	2	23 (31.5)	13 (19.4)	
	3	18 (24.7)	8 (11.9)	
	4 or more	17 (23.3)	6 (9.0)	
Albumin ≤24.5 g/L	unknown	2 (2.7)	4 (6.0)	
	no	25 (34.2)	28 (41.8)	0.29
	yes	46 (63.0)	35 (52.2)	
WCC >12×10^9/L	unknown	0 (0.0)	1 (1.5)	
	no	46 (63.0)	51 (76.1)	0.1
	yes	27 (37.0)	15 (22.4)	
Respiratory rate >17 breaths per minute	no	21 (28.8)	48 (71.6)	<0.0001 (phi=0.43)
	yes	52 (71.2)	19 (28.4)	
CRP >228 mg/L	unknown	0 (0.0)	12 (17.9)	
	no	64 (87.7)	52 (77.6)	0.23
	yes	9 (12.3)	3 (4.5)	
Temp >38.5 °C	no	51 (69.9)	54 (80.6)	0.17
	yes	22 (30.1)	13 (19.4)	
Raised serum creatinine (>90µmol/L)	no	40 (54.8)	47 (70.1)	0.08
	yes	33 (45.2)	20 (29.9)	

Figure 3.1 illustrates a greater number of severity indicators present in the non-survivors as compared to the survivors. Therefore, these severity indicators may be synonymous with mortality indicators for CD infection.

Figure 3.1: Comparison of survivors and non-survivors of *Clostridium difficile* in terms of the number of severity indicators in each group

3.10 Comparison of HIV-positive and HIV-negative patients with *Clostridium difficile* infection

Table 3.15 demonstrates the comparison of HIV-negative and HIV-positive patients with *Clostridium difficile* infection in terms of demographics and clinical presentation. With regards to the HIV-negative patients, 44% were 65 years and older as compared to none of the HIV-positive patients ($p < 0.0001$; phi coefficient = 0.6). A higher proportion of HIV-negative patients (13%) had bloody diarrhoea as compared to only 2% of the HIV-positive patients ($p = 0.016$; phi coefficient = 0.23).

Table 3.15: Comparison of HIV-positive and HIV-negative patients with *Clostridium difficile* infection in terms of demographics and clinical presentation (signs and symptoms).

Variable	**Category**	**Overall n (%)**	**HIV-negative n (%)**	**HIV-positive n (%)**	**p-value**
N		**154**	**39**	**105**	
Gender	Female	83 (53.9)	19 (48.7)	59 (56.2)	0.46
	Male	71 (46.1)	20 (51.3)	46 (43.8)	
Age	<65 years	134 (87.0)	22 (56.4)	105 (100.0)	<0.0001 (phi=0.6)
	≥65 years	20 (13.0)	17 (43.6)	0 (0.0)	
Clinical presentation (symptoms and signs)	Watery diarrhoea	143 (92.9)	36 (92.3)	99 (94.3)	0.7
	Bloody diarrhoea	9 (5.8)	5 (12.8)	2 (1.9)	0.016 (phi=0.23)
	Fever (>38°C)	42 (27.3)	7 (17.9)	33 (31.4)	0.14
	Abdominal pain	25 (16.2)	7 (17.9)	16 (15.2)	0.8
	Nausea and/or vomiting	18 (11.7)	2 (5.1)	14 (13.3)	0.24
	Loss of appetite	16 (10.4)	1 (2.6)	15 (14.3)	0.07
	Peritonism	1 (0.6)	0 (0.0)	1 (1.0)	>0.99
	Ileus	3 (1.9)	0 (0.0)	2 (1.9)	>0.99
	Other	2 (1.3)	1 (2.6)	1 (1.0)	0.47

Table 3.16 demonstrates the comparison of HIV-negative and HIV positive patients in terms of medications as risk factors for *Clostridium difficile.* All of the HIV-positive patients had been exposed to antibiotics within the last 30 days as compared to 92% of the HIV-negative patients (p = 0.018; phi coefficient = 0.25). There was no difference between the exposure to each individual antibiotic in HIV-negative and HIV-positive patients. There was no significant difference in the exposure to opioids, immunosuppressives, diclofenac or antidepressants between HIV-negative and HIV-positive patients. Overall, 51% of the HIV-negative patients had been exposed to gastric acid suppressants as compared to a smaller proportion (21%) of HIV-positive patients (p = 0.008; phi coefficient = 0.3). In particular, exposure to intravenous PPIs was higher amongst HIV-negative patients (75%), compared to HIV-positive patients (36%) (p = 0.016; phi coefficient = 0.39).

Table 3.16: Comparison of HIV-positive and HIV-negative patients with *Clostridium difficile* infection in terms of medications as risk factors

Risk factor	**Category**	**Overall n (%)**	**HIV-negative n (%)**	**HIV-positive n (%)**	**p-value**
N		**154**	**39**	**105**	
Antibiotics in prior 30 days	Unknown	6 (3.9)	2 (5.1)	3 (2.9)	
	No	3 (1.9)	3 (7.7)	0 (0.0)	0.018 (phi = 0.25
	Yes	145 (94.2)	34 (87.2)	102 (97.1)	
Gastric acid suppression	No	106 (68.8)	19 (48.7)	83 (79.0)	0.008 (phi = 0.30
	Yes	48 (31.2)	20 (51.3)	22 (21.0)	
Oral PPI	No	22(45.8)	13 (65.0)	8 (36.4)	0.12
	Yes	26 (54.2)	7 (35.0)	14 (63.6)	
IV PPI	No	24 (50.0)	5 (25.0)	14 (63.3)	0.016 (phi = 0.39)
	Yes	24 (50.0)	15 (75.0)	8 (36.4)	
Opioids	No	119 (77.3)	29 (74.4)	83 (79.0)	0.65
	Yes	35 (22.7)	10 (25.6)	22 (21.0)	
Immunosuppressives	No	100 (64.9)	29 (74.4)	63 (60.0)	0.12
	Yes	54 (35.1)	10 (25.6)	42 (40.0)	
Anti-depressants	No	154 (100.0)	39 (100.0)	105 (100.0)	
	Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Loperamide	No	153 (99.4)	39 (100.0)	104 (99.0)	>0.99
	Yes	1 (0.6)	0 (0.0)	1 (1.0)	
Diclofenac	No	154 (100.0)	39 (100.0)	105 (100.0)	
	Yes	0 (0.0)	0 (0.0)	0 (0.0)	

PPI = proton pump inhibitor, IV = intravenous

Table 3.17 indicates the comparison of the types of severity indicators and the number of severity indicators in HIV-positive and HIV-negative patients with *Clostridium difficile* infection. Overall, HIV-negative patients tended to have a higher number of severity indicators compared to HIV-positive patients ($p = 0.011$; Cramer's $V = 0.32$). A higher proportion of HIV-negative patients (61.5%) had an elevated WCC compared to HIV-positive patients (17.1%) ($p < 0.0001$; phi coefficient = 0.43). Similarly, a higher number of HIV-negative patients had an elevated respiratory rate (69.2%) compared to HIV-positive patients (43.8%) ($p = 0.0085$; phi coefficient = 0.23). HIV-negative patients also more

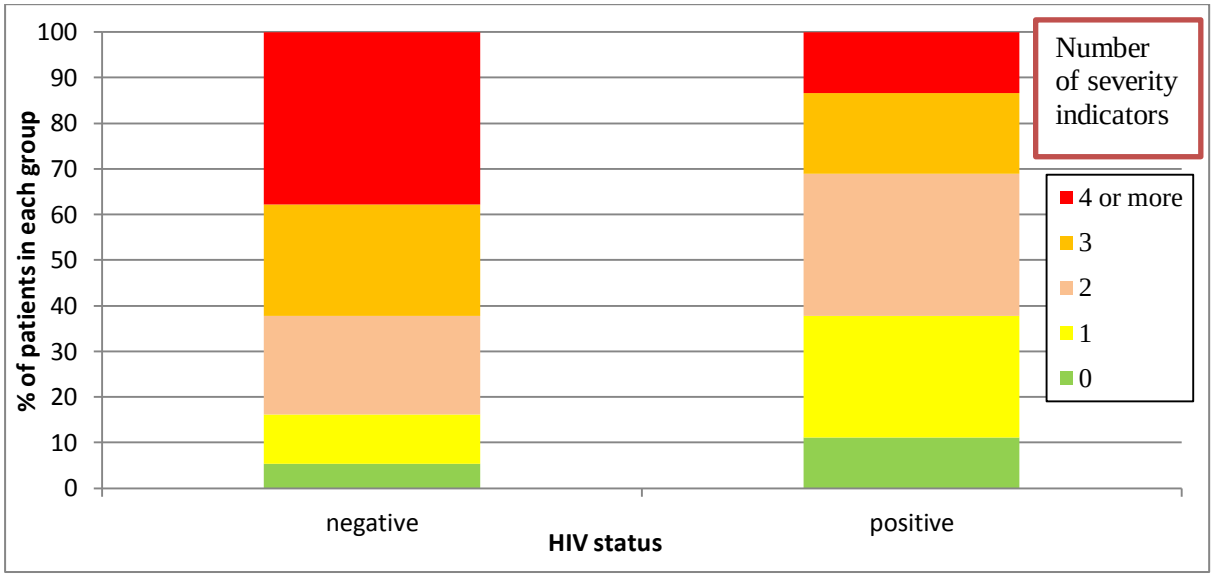
commonly had elevated temperatures (41%) compared to HIV-positive patients (19%) (p=0.0095; phi coefficient = 0.23).

Table 3.17: Comparison of HIV-positive and HIV-negative patients with *Clostridium difficile* infection in terms of type and number of severity indicators

Severity indicator	**Category**	**Overall n (%)**	**HIV-negative n (%)**	**HIV-positive n (%)**	**p-value**
N		**154**	**39**	**105**	
Number of severity indicators present	Unknown	18 (11.7)	2 (5.1)	15 (14.3)	
	0	13 (8.4)	2 (5.1)	10 (9.5)	0.011 (V=0.32)
	1	31 (20.1)	4 (10.3)	24 (22.9)	
	2	38 (24.7)	8 (20.5)	28 (26.7)	
	3	27 (17.5)	9 (23.1)	16 (15.2)	
	4 or more	27 (17.5)	14 (35.9)	12 (11.4)	
Albumin ≤24.5 g/L	unknown	7 (4.5)	1 (2.6)	6 (5.7)	
	no	56 (36.4)	15 (38.5)	35 (33.3)	0.69
	yes	91 (59.1)	23 (59.0)	64 (61.0)	
WCC >12×10^9/L	unknown	1 (0.6)	0 (0.0)	1 (1.0)	
	no	106 (68.8)	15 (38.5)	86 (81.9)	<0.0001 (phi=0.43)
	yes	47 (30.5)	24 (61.5)	18 (17.1)	
Respiratory rate >17 breaths per minute	no	77 (50.0)	12 (30.8)	59 (56.2)	0.0085 (phi=0.23)
	yes	77 (50.0)	27 (69.2)	46 (43.8)	
CRP >228 mg/L	unknown	13 (8.4)	1 (2.6)	11 (10.5)	
	no	126 (81.8)	35 (89.7)	82 (78.1)	0.55
	yes	15 (9.7)	3 (7.7)	12 (11.4)	
Temp > 38.5 °C	no	117 (76.0)	23 (59.0)	85 (81.0)	0.0095 (phi=0.23)
	yes	37 (24.0)	16 (41.0)	20 (19.0)	
Raised serum creatinine (>90µmol/L)	no	98 (63.6)	22 (56.4)	70 (66.7)	0.33
	yes	56 (36.4)	17 (43.6)	35 (33.3)	

Figure 3.2 illustrates the greater number of severity indicators present in HIV-negative patients with *Clostridium difficile* infection.

Figure 3.2: Comparison of HIV-negative and HIV-positive patients with *Clostridium difficile* infection in terms of number of severity indicators in each group



Overall, there was no significant difference in survival of HIV-negative and HIV-positive patients with *Clostridium difficile* infection. This is demonstrated in table 3.18.

Table 3.18: Comparison of outcomes in HIV-positive and HIV-negative patients with *Clostridium difficile* infection

Outcome	**Category**	**Overall n (%)**	**HIV-negative n (%)**	**HIV-positive n (%)**	**p-value**
N		**154**	**39**	**105**	
Outcome	Unknown (includes transfer and RHT)	14 (9.1)	3 (7.7)	9 (8.6)	
	Died	73 (47.4)	22 (56.4)	49 (46.7)	0.33
	Survived	67 (43.5)	14 (35.9)	47 (44.8)	

4.0 DISCUSSION

In general, the major findings of the study are as follows. The study population was younger than the expected age of patients at risk for CD infection. The patients commonly presented with the usually described symptoms and signs of CD infection in particular, diarrhoea and fever. Patients also demonstrated the commonly known risk factors for CD infection including: exposure to antibiotics in the prior 30 days and exposure to gastric acid suppressants (in particular PPIs), immunosuppressants or opioids. Patients often had one or more previous hospital admissions (42.2% of patients) and often had prolonged hospital stays. There was a clear predominance of HCAIs with only two identifiable cases of community-acquired CD infection. The most commonly identified severity indicator was an albumin of ≤24.5g/L and non-survivors tended to have a greater number of severity indicators. The overall mortality of patients with CD infection in this study is significantly higher than expected as overall 47.4% of patients died. Survivors were most commonly treated with metronidazole 400mg administered 8 hourly for a duration of more than 10 days. When comparing HIV-positive patients with HIV-negative patients, the latter patients tended to have more bloody diarrhoea and more exposure to gastric acid suppressants in particular, intravenous PPIs. HIV-negative patients tended to have a greater number of severity indicators and more commonly had an elevated WCC, respiratory rate and temperature.

The age of the patients was lower than has been described in some previous studies. The median age in this study was 39 years which is the same as the median age in the South African study by Rajabally et al. (2013) but much younger than the mean age of 70.8 years in the Canadian study by Oake et al. (2010). Overall only 13% of patients were found to be older than 65 years which is lower, than expected since age above 65 years is considered a risk factor for CD infection (Bobo, Dubberke & Kollef 2011). This younger median age in

the South African studies is almost certainly due to the large number of HIV-positive patients within the study populations.

The patients in the current study were exposed to several well known risk factors for CD infection such as antibiotic use in the prior 30 days. This risk factor was present in 97.6% of study patients, making it by far the most common risk factor. This begs the question as to whether the antibiotics were truly warranted in these patients in the first place considering the risk of CD infection. Although some patients were exposed to the common antibiotic culprits such as the fluoroquinolones, cephalosporins and clindamycin (Bobo, Dubberke & Kollef 2011), the most common antibiotics that patients had been previously exposed to in this study were amoxicillin-clavulanic acid and piperacillin-tazobactam. Regardless of the antibiotic type used, it is essential for clinicians to have a more circumspect approach when prescribing antibiotics to patients since their use is not without significant risks.

Patients commonly had previous hospital admissions within the prior 90 days and the median number of days admitted was 17 days. Only 18 (11.6%) patients had been admitted for less than 7 days. This mirrors the study by Oake et al. (2010) which demonstrated that the majority of hospital-acquired CD is identified after the 7th day of hospital admission.

In this study, 47.4% of patients died. This reflects a significantly higher mortality rate than the reported 20% in the Cape Town study by Rajabally et al. (2013) and the study by Mitchell and Gardner (2012), which reported in-hospital mortality rates ranging between 8 and 37%. The reason for this increased mortality rate at the CMJAH is unknown and further studies would be required to assess possible causes. While it may be interesting to suggest that the high mortality rate may be due to the high prevalence of HIV infection in the current study population, as indicated below, the mortality in HIV-positive and HIV-negative cases was not significantly different.

When comparing survivors and non-survivors in the current study, there were no significant differences in the demographics, clinical presentation or the risk factors present. Age above 70 years has been associated with increased mortality (Bobo, Dubberke & Kollef 2011), however this was not apparent in this study due to the low median age of the study population and the few numbers of patients that were of that age. Corticosteroid use has been shown to increase short-term mortality in hospitalised patients with CD infection, however this was not found in the current study (Gupta & Khanna 2014).

The survivors in this study had more commonly been treated with oral metronidazole 400mg 8 hourly than the non-survivors. This does not necessarily indicate that there is an improved outcome of CD infections with the use of oral metronidazole but may simply indicate that patients who were given oral metronidazole were likely to be those who only had mild to moderate disease in whom better outcomes may have been predictable from the start. Furthermore, a greater number of non-survivors were treated with a combination of oral and intravenous metronidazole compared to the survivors. Again, this may not necessarily reflect a poorer outcome with this treatment regimen but may indicate patients with severe disease and increased severity who were given a combination of oral and intravenous therapy, and had a poorer prognosis from the start. As expected, the non-survivors tended to have a greater number of severity indicators which supports the findings of Butt et al. (2013). Therefore, these severity indicators could also be referred to as mortality indicators.

It is important to note that none of the patients in this study were treated with metronidazole 500mg administered 8 hourly as recommended by guidelines (Surawicz et al. 2013); instead the dose used was 400mg administered 8 hourly. This was likely because this is the formulation of metronidazole available at this hospital. Further studies would be required to determine if this small change in dosage has any implication on clinical response or outcome. The patients with ileus, toxic megacolon and pseudomembranous colitis were not managed

with rectally administered vancomycin as recommended. Some patients however, did receive high doses of vancomycin (with intravenous metronidazole). A dose commonly administered was 250mg 6 hourly rather than 500mg 8 hourly of vancomycin. The survivors were more commonly treated for longer as compared to the non-survivors. However, this may be the case because many patients died before the treatment was completed. It was noted that in most cases, treatment for CD infection was initiated whilst continuing other antibiotics. It is usually recommended that antibiotics other than metronidazole or vancomycin be stopped however; this may not have been possible due to the ongoing treatment of other concomitant life-threatening infections (Collini et al. 2013).

Furthermore, in 14.3% of patients, the medical records did not indicate any evidence of the patients having been treated for CD infection. Possible reasons for this may be due to death, discharge or refusal of hospital treatment among the patients prior to initiation of treatment for CD infection. Another possibility may be lack of follow up of results of stool specimens sent. As indicated above the overall mortality rate of the study population was 47.4% being 54.5% in the untreated patients and 45.7% in the treated patients. This was not significantly different. However it cannot be determined whether any of these deaths were solely due to CD disease.

Interestingly, only two patients (1.3%) were identified as having community-acquired CD infection. This is much lower than the 32% found in a previous South African study (Rajabally et al. 2013) and the 20 to 50 cases per 100 000 population in the United States, Sweden and England (Chitnis et al. 2013). The reasons for this are uncertain. It is possible that this may be due to community-acquired CD infections being much milder, and therefore these patients would be treated as out-patients and not in a tertiary hospital such as CMJAH; however the current study only investigated CD infection in the hospital setting.

Overall 68.1% of patients were HIV-positive and only 46.7% of these patients were on anti-retroviral therapy. The median CD4 cell count of the HIV-seropositive group was 59 ($x10^6$/L). This may demonstrate the apparent susceptibility of HIV-positive patients with low CD4 cell counts to CD infection presumably due to their abnormal humoral or cell-mediated immunity and altered gut microbiome as described by Raines and Lopez (2013). These patients are also more likely to have recurrent opportunistic infections, requiring frequent contact with the healthcare system and recurrent antibiotic exposure. Those authors suggested that the risk of CD in HIV-positive patients was twice that of HIV-negative patients. According to Statistics South Africa, the prevalence of HIV-positive patients in the country during 2013 and 2014 was 18.7% and 18.8% respectively (Statistics South Africa 2014). As such, if HIV infection was not a risk factor for CD infection, we would have expected a similar rate of HIV infection in our study population. However, the prevalence of HIV-positive patients in the current study was 68.1%. This may suggest that the risk of CD infection in HIV-positive patients is more than 3.5 fold higher than that of HIV-negative patients in this study. However, this may also simply be a reflection of the higher HIV prevalence among hospital in-patients.

More than half of the current study patients were on TB therapy; however this may be reflective of the types of patients seen in the specific wards assessed in this study rather than it being a risk factor for acquiring CD. In this regard, ward 487 and 587 are infectious diseases wards to which TB patients (especially sputum positive TB patients) are mainly admitted.

When comparing the HIV-positive patients with CD to the HIV-negative patients, as mentioned before, HIV-negative patients more commonly had bloody diarrhoea. HIV-negative patients were also more commonly exposed to gastric acid suppressants, and in particular intravenous PPIs. All of the HIV-positive patients were exposed to antibiotics

within the prior 30 days compared to 92% of HIV-negative patients. There was no difference in the clinical presentation or outcomes of HIV-positive and HIV-negative patients which is similar to the findings of by Raines & Lopez (2011). HIV-negative patients had a greater number of severity indicators and more commonly had elevated WCC (>12 × 10^9/L), respiratory rates (>17 breaths per minute) and temperatures (>38.5°C). This could possibly be explained by their ability of HIV-negative patients to mount a better immune response against the infection than HIV-positive patients.

This study has some potential limitations. Firstly, the study was retrospective in design and therefore not all data that we wished to collect may have been available. However, patients whose files were missing, or those who had substantial data missing from their files were excluded. Fortunately most of the records of the patients in this study could easily be traced. The study was a single-centre study selecting only patients admitted to a tertiary hospital, with a large proportion of patients coming from the infectious diseases wards. This may have biased the patient profile and characteristics and therefore it is not clear whether the findings are generalizable to the general population. The strengths of this study lie in its successful retrieval of most clinical data required and its large sample size which allowed for comparisons to be made between the survivors and non-survivors and the HIV-seropositive and HIV-seronegative groups.

In future it would be of benefit to include out-patients as well as in-patients with CD infection in the hope of capturing more patients with community-acquired CD infections. This would allow for comparisons between community-acquired and healthcare-associated infections in order to better understand the characteristics of community-acquired CD infections. It would also be beneficial to include a population outside of the infectious diseases wards (perhaps including surgical wards) to broaden the patient profile. As mentioned previously, further

studies would be required to investigate possible causes for the high mortality rate found in this study.

5.0 CONCLUSION

In general, the patients in this study with CD infection were younger than those described in non-South African studies, but comparable to that of other South African studies. The mortality of patients with CD was found to be much higher in Charlotte Maxeke Hospital; the reason which is currently unknown. The study solidified and reinforced evidence for the previously known risk factors for CD infection. These risk factors included: exposure to antibiotics, immunosuppressives, gastric acid suppressants, opioids and multiple or prolonged admissions. Similarly, patients presented with common signs and symptoms and there was no difference in the clinical presentation of the survivors and non-survivors or the HIV-positive and HIV-negative patients. There were only two identifiable cases of community-acquired CD infection which is much lower than what has been reported in previous local and international studies. WCC $>12\times10^9$/L, albumin $\leq$24.5g/L, temperature >38.5°C, respiratory rate >17 breaths per minute and raised serum creatinine (>90µmol/L) were found to be important features of severity that could be used to predict mortality. The non-survivors had greater number of severity indicators than the survivors. There was no difference in the outcomes of HIV-positive and HIV-negative patients. This study paves the way for further investigation as there as still many unanswered questions regarding the characteristics of patients with community-acquired CD infections and the reasons for the increased mortality at this Johannesburg hospital.

Considering the high mortality of the disease, practicing clinicians need to be aware of risk factors for this infection, avoiding them where possible. Effective antibiotic stewardship is essential to avoid unnecessary exposure of patients to antibiotics. Furthermore, the presence of characteristic symptoms should prompt early diagnosis of CD infection in high risk patients with early initiation of appropriate treatment.

6.0 APPENDIX A

Study Number: _____

Demographics

Gender	Male	
	Female	

Age	Value	
	≥65 years	
	<65 years	

Symptoms/signs present

Watery diarrhoea	
Bloody diarrhoea	
Fever	
Abdominal pain	
Nausea and/or vomiting	
Loss of appetite	
Peritonism/acute abdomen	
Ileus	
Other	

Medications as risk factors

Exposure to antibiotics in the prior 30 days	No		Yes		Unknown	
			Amoxicillin-clavulanic acid			
			Piperacillin-tazobactam			
			Ertapenem			
			Imipenem			
			Meropenem			
			2nd Generation cephalosporin			
			3rd Generation cephalosporin			
			4th Generation cephalosporin			
			Fluoroquinolone			
			Clindamycin			
			Cloxacillin			
			Vancomycin			
			Other			
			Unknown			

Gastric acid suppression	No		Yes		Unknown	
			Antacid			
			H2 rec antagonist			
			Oral PPI			
			IV PPI			
Diclofenac	No		Yes		Unknown	
Opioids	No		Yes		Unknown	
			Codeine?			
			Pethidine?			
			Morphine?			
			Tramadol?			
Immunosuppressive drugs	No		Yes		Unknown	
			Steroid			
			IV?			
			Oral?			
			Other?			
Mirtazepine/nortryptiline/fluoxetine	No		Yes		Unknown	
Loperamide	No		Yes		Unknown	

Other medications

	No	Yes	Unknown
On TB treatment			
On anti-retrovirals			

HIV

HIV	Negative	Positive	Unknown

Community-acquired infection or healthcare-associated infection

Previously admitted?	Yes		No		Unknown	
	>90 days ago					
	≤90 days ago					
	Duration					
	<2 days					
	≥2 days					

Current admission	Number of days	Unknown
Duration of hospital stay	Ward:_____	
Duration of ICU stay		

Healthcare-associated infection		Community-acquired infection	Unknown
Admitted in last 90 days			
Nosocomial (>48hrs after admission)			
Nursing home			
Home-based care			
Long-term care facility			
Health care worker			
Out-patient – chemotherapy/dialysis			

Treatment

Drug	Yes	Dose and duration
Oral Metronidazole		
Intravenous Metronidazole		
Oral and IV Metronidazole		
Oral Vancomycin		
Other		Which other?
Unknown		

Indicators of severity

	Yes	No	Value	Unknown
Albumin ≤24.5 (g/L)				
WCC>12 ($x10^9$/L)				
Respiratory rate>17 (bpm)				
CRP>228 (mg/L)				
Temperature>38.5 (°C)				
Raised serum creatinine (µmol/L)				

Other blood parameters

	Value	Unknown
WCC ($x10^9$/L)		
Hb (g/dL)		
Platelets ($x10^9$/L)		
Sodium (mmol/L)		
Creatinine (µmol/L)		
Albumin (g/L)		
CD4 count (if HIV positive)		
HIV viral load (if HIV positive)		

Outcome

Outcomes	Death	Survival (discharge)	Unknown
			Transfer
			Signed RHT
			Outcome unknown

7.0 APPENDIX B

R14/49 Dr Lee-Anne Godinho

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M141028

NAME: (Principal Investigator)	Dr Lee-Anne Godinho
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Clostridium Difficile at Charlotte Maxeke Johannesburg Academic Hospital: A Descriptive Study
DATE CONSIDERED:	31/10/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Charles Feldman
APPROVED BY:	Professor Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	10/11/2014

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 Secretary in Room 10004, 10th floor, Senate House, University.
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**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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