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To ascertain the prevalence of clostridium difficile infection in cohort of HIV positive patients with diarrhoea at Chris Hani Baragwanath academic hospital

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TO ASCERTAIN THE PREVALENCE OF *CLOSTRIDIUM DIFFICILE* INFECTION IN A COHORT OF HIV POSITIVE PATIENTS WITH DIARRHOEA AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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A research report submitted to the faculty of Health Sciences, University of the Witwatersrand, in partial fulfillment of requirement for degree of Master of Medicine in Internal Medicine

DECLARATION

I, T.P Shabangu, declare that this research report is my own work. It has not been submitted for any degree examination other than for the degree of Masters of Medicine

Thulisani Phillipine Shabangu

This …..Day of ……..2016

DEDICATION

My patients who have awarded me an opportunity to serve them

ABSTRACT

Introduction:

Clostridium difficile infection (CDI) affects the digestive system; the symptoms range from mild to severe. In healthy individuals CDI is asymptomatic; however certain antibiotics and other medication can disturb the normal gut flora predisposing to CDI. This may lead to unnecessary hospitalisation or a prolonged hospital stay, which can be more debilitating in immunocompromised patients. Thus, judicious antibiotic use is crucial; however certain conditions require treatment that may alter normal flora, which is a predisposing factor for CDI.

Objective: To ascertain the prevalence of *Clostridium difficile* infection in a cohort of HIV positive patients with diarrhoea at Chris Hani Baragwanath Academic Hospital. (CHBAH) over a 12 month period.

Design: This was a prospective study.

Methods:

- Prospective study, at CHBAH
- 200 HIV positive patients with diarrhoea were evaluated.
- Clinical records of the selected patients were accessed.
- A questionnaire was used to identify risk factors for *Clostridium difficile* infection (CDI) in the selected patients.
- Stool analysis was used to diagnose CDI.

Results:

Fifty-three patients (26.5%) had CDI.

The most significant factors associated with an increased risk for CDI were:

- Anti-tuberculous treatment; most likely Rifampicin
- Antibiotic use, especially penicillin based drugs; clindamycin and carbapenems.

A very low CD4 count was not a strong predictor for CDI (p=0.62) after adjusting for confounders (Viral load, concurrent co-morbid disease, use of antibiotics and anti-tuberculosis drugs).

Conclusions:

In our cohort of 200 patients, fifty-three (26.5%) had CDI. The risk factors identified were use of anti- TB drugs, common antibiotics associated with *C.difficile*.

ACKNOWLEDGEMENTS

I would like to acknowledge the tremendous support and guidance from my supervisor, Prof Ally. Your knowledge and humble spirit has left my heart with a smile.

I would to also acknowledge the assistance of Dr. K. Hari

The internal medicine team who informed me about patients and CHBAH management for allowing me access to their sick patients.

The statistician: Dr Mphatso Kamndaya

Finally my patients for their co-operation and kindness, I would not have done this without them.

ABBREVIATIONS

AAD	Antibiotic–associated diarrhea
ADP	Adenosine diphosphate
C.	*Clostridium*
CDAD	*Clostridium difficile*-associated diarrhea
CDI	*Clostridium difficile* infection
CI	Confidence interval
CHBAH	Chris Hani Baragwanath Academic Hospital
GTP	Glutamyltranspeptidase
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
NAP 1	American pulsed field type 1 (NAP1)
OR	Odds ratio
PCR	Polymerase chain reaction
PMC	Pseudomembranous colitis
TB	Tuberculosis

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1.0 CHAPTER 1

1.1 Introduction and literature review

1.1.1 *Clostridium difficile*

Clostridium difficile (*C.difficile*) is a Gram-positive rod shaped bacterium of the genus *Clostridium*, it exists in vegetative or spore forms. The antibiotics can eradicate the normal gut flora leading to CDI presenting with diarrhoea and gastrointestinal complications. (1). Scientific classification of *C. difficile* is shown in table 1.1 below

Table 1.1: Scientific classification of *C. difficile*

Kingdom	Bacteria
Phylum	Firmicutes
Class	Clostridia
Order	Clostriadiales
Family	Clostridiaceae
Genus	*Clostridium*
Species	*C. difficile*

(2)

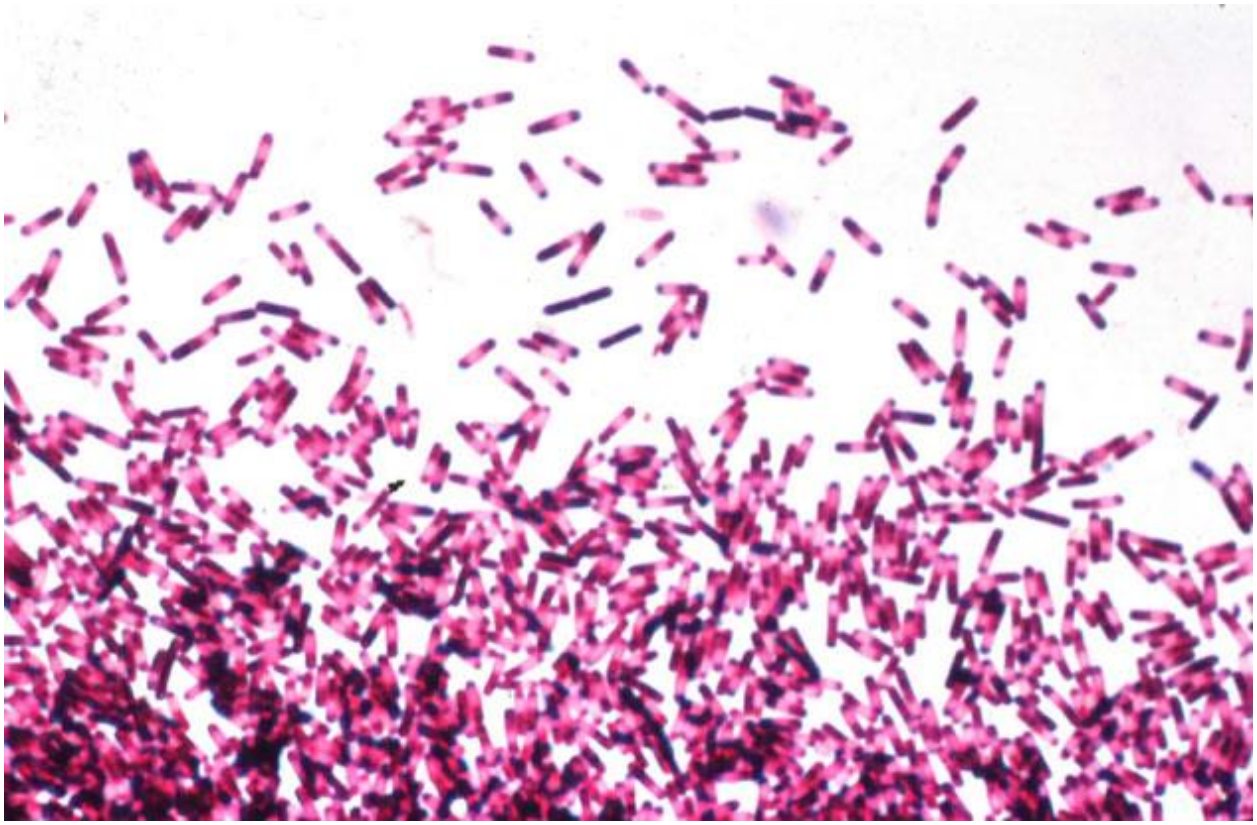


Figure 1.1: *Clostridium difficile*

(3)

Clostridia bacteria are anaerobic gram positive rods which forms spores. Its first description was in 1935, and at that stage, it was not thought of as a pathogen but as the normal faecal flora of healthy newborns. It is known to cause antibiotic–associated diarrhoea (AAD) because the antibiotics destroy the normal gut microbiota leading pseudomembranous colitis (PMC). This was elucidated around 1970(1, 4)

C.difficile secretes two types of exotoxin strains which are toxin A (TcdA an enterotoxin) and toxin B (TcdB a cytotoxic).Production of the above mentioned strains results in the disease and the prognosis depends on immunity of the host and toxin strain virulence produced. The clinical manifestations of CDI can vary from

asymptomatic presentation to severe diarrhea, PMC, toxic megacolon, and colonic perforation (1, 4, 5).

Dating back about ten years ago, there is a significant rise in the occurrence of CDI and the complications of *Clostridium difficile*-associated diarrhea (CDAD). This is partly attributable to the appearance of the new strain, known as North American pulsed field type 1 (NAP1).(6).There is a difference between *Clostridium difficile* colonisation and *Clostridium difficile* infection the former exhibits no symptoms even though patients have a positive test for organism and/or toxins. Colonisation is more common than infection. Patients with infection will exhibit symptoms secondary to the organism and/toxins (7, 8).

1.2 Epidemiology of *Clostridium difficile* infection

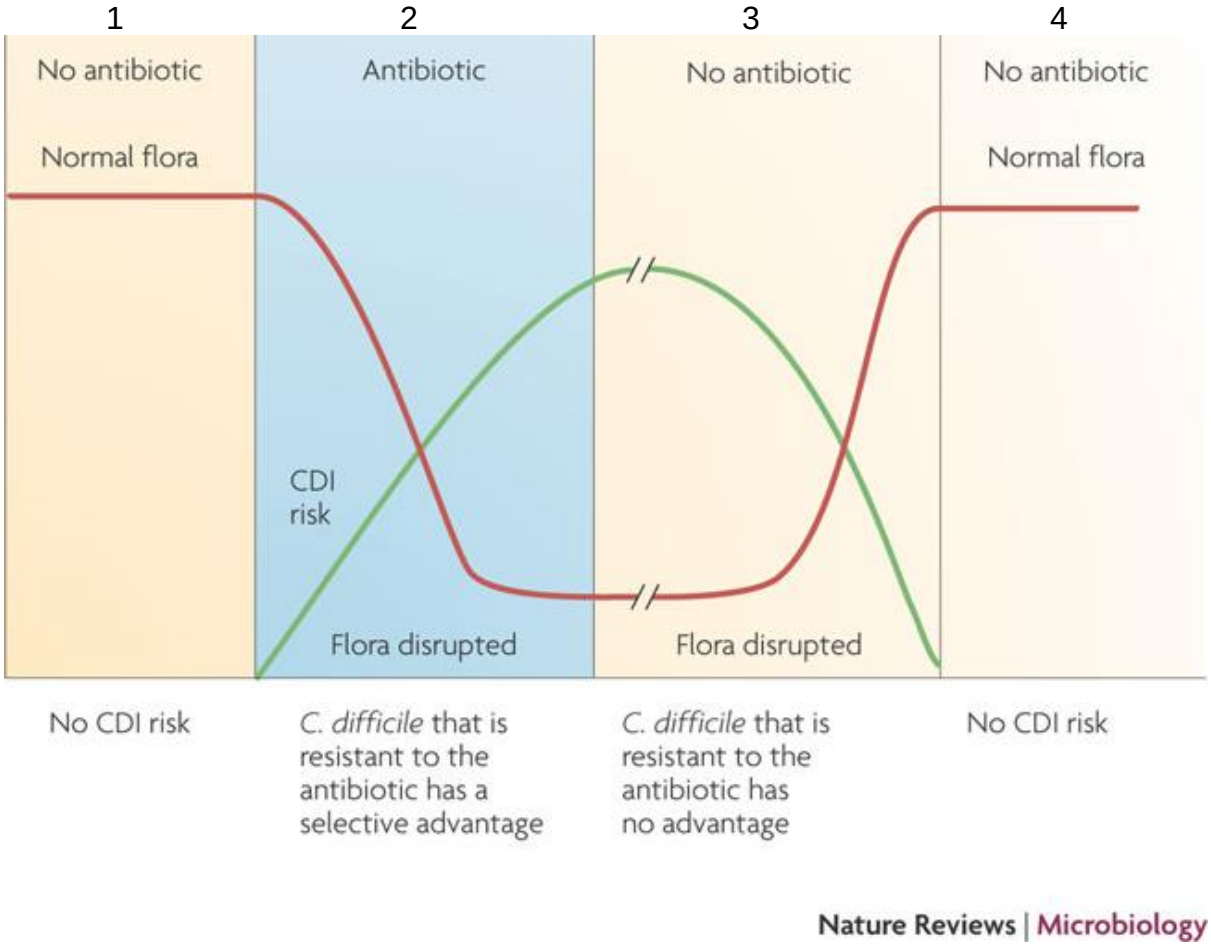
Previous research done at America, more than 44,000 HIV positive patients were recruited from 1992-2002, the most common aetiology of the diarrhoea was found to be *C.difficile* (9).

About fourteen years ago they discovered an epidemic strain leading to the rise of the occurrence of CDI and its complications. (10).

Diarrhoea is one of the most common presentations in HIV positive patients with more than 50% of these patients presenting with diarrhoea during course of their illness. Approximately 75 % of HIV positive patients will experience morbidity and mortality related to diarrhoea. In 50% of cases, HIV positive patients presenting with diarrhoea have no identifiable aetiology. It is then suggested that the symptoms are attributed HIV enteropathy (12)

1.3 Pathogenesis

C.difficile produces toxins and spores that are unaffected by heat and can survive in the environment for long. It is a normal commensal of the gut. The gut microbiota prevents overpopulation of *C.difficile* (1, 4-6, 11, 12). Antibiotics administration can change the normal gut microbiota predisposing patients to antibiotic associated diarrhea (AAD) .*C.difficile* is the most significant cause of AAD which can complicate to PMC. This is because *C.difficile* produces exotoxin that destroys mucosa by causing colon inflammation. An impaired barrier in the epithelial function has been described, it is postulated that diarrhoea by leak flux mechanism is secondary to destruction of the tight junctions as a result of HIV chronic inflammation states that produce cytokine. Denervation of anatomy, dysregulation of immune system, malabsorption of bile acid and local production of lymphokines are other mechanism proposed.(1,4-7)



Patients will not get CDI if their normal gut flora is not disrupted by antibiotics (1). Once antibiotic treatment is initiated, CDI with strain resistance to antibiotics is more likely while the antibiotic is being administered because of presence of antibiotic in the gut (2). When the antibiotic treatment is withdrawn, the antibiotic levels in the gut drops significantly and rapidly, however the gut flora remains disturbed for some time as indicated by the break in the graph, this depends on the type of antibiotic given (3). During this time, patients can be infected with C.difficile .When the microflora recovers, colonization resistance to C. difficile is restored (4).

Figure 1.2: *C-difficile* and antibiotic pathogenesis

(13)

When common antibiotics associated with CDI have been administered, the gut flora will be disrupted exposing gut to be overrun by *C.difficile* organism leading to CDI. This overpopulation results in symptoms of CDI. The feared complication *C.difficile* is

pseudomembranous colitis, which has high mortality especially if it progress to toxic megacolon (10).

Several toxins are produced by *C.difficile* strains. Tcd A and B can both cause diarrhoea and inflammatory response, however their relative contributions have been debated (1, 14).

It is described in literature that the Rho family of GTPases can be inactivated by toxins A and B are glucosyltransferases. Actin depolymerisation is induced by exo-toxins following a decrease in the ADP-ribosylation of the low molecular mass glutamyltranspeptidase (GTP)-binding Rho proteins. The toxins that are bound to receptors can enter intracellularly and catalyse specific alterations of Rho proteins, small (GTP)-binding proteins that helps in structure of cytoskeleton, polymerisation of actin, and movements of the cell. Binary toxin has been previously researched but its mechanism is still under development (14).

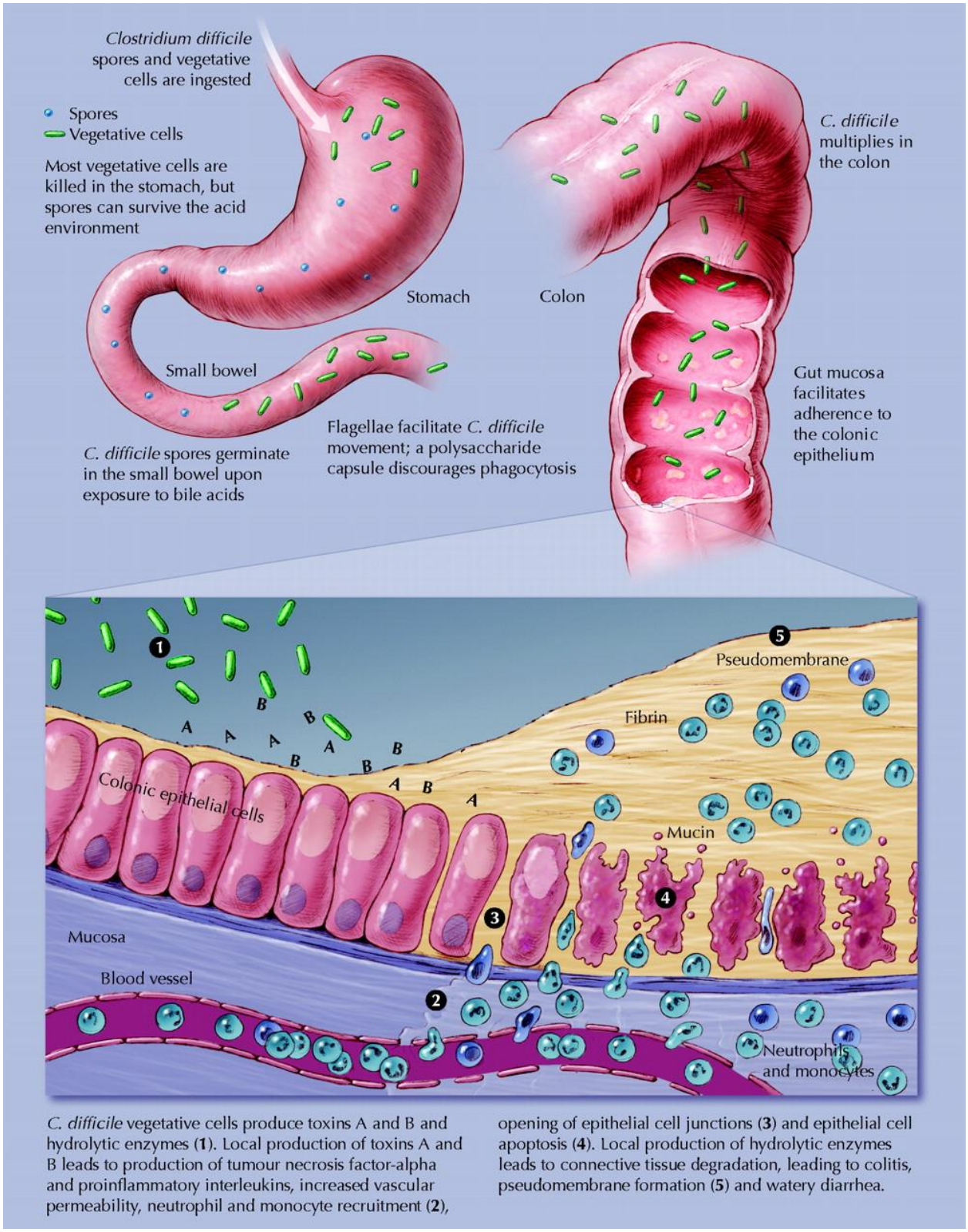


Figure 1.3: Pathogenesis of *C.difficile*.

(15)

1.4 Risk Factors

- Antibiotic exposure:
    - Penicillin based drugs (amoxicillin, ampicillin, augmentin)
    - Clindamycin
    - Carbapenems
    - Cephalosporin (especially second and third generation)
    - Less commonly implicated: protein synthesis inhibitors (erythromycin, azithromycin, clarithromycin) (16, 17)
- Proton pump inhibitors
- Antineoplastic agents
- Hospitalisation:
    - Prolonged stay
    - Intensive Care Unit
    - Sharing room with infected person if strict infection control measures neglected
- Advanced age
- Malignancies
- Necrotising colitis
- Inflammatory bowel disease
- Renal failure

1.5 Transmission

C. difficile is found in the stool. Virtually anything that can be contaminated with infected stool can serve as a main entry for *C. difficile* spores that are then transferred to patients, mainly via the contaminated. (1)

1.6 Clinical features

Patients with *C. difficile* infection may present with the following symptoms and signs. (1)

Mild to moderate or severe diarrhoea

Cramping abdominal pain

Anorexia

Malaise

Fever

These symptoms may mimic inflammatory bowel disease associated colitis

The clinical features of C. difficile infection vary from asymptomatic to diarrhoea with severe colitis

Table 1.2: Clinical manifestations of *Clostridium difficile* infection

Type of infection	Diarrhea	Other symptoms	Physical examination	Sigmoidoscopic examination
Diarrhea with colitis	≥loose stool faecal white cell count Occult bleeding Hematochezia (uncommon)	Nausea, loss of appetite, fever, fatigue, dehydration, leukocytosis with left shift	Tenderness and distention of abdomen	Non -specific colitis
Severe colitis	Diarrhea varies from severe to diminished	Pyrexia,tachycardia, lerthagic and abdominal pain	peritonitis	Only proctoscpy recommended

	because of paralytic ileus and megacolon			
Asymptomatic carriage	Nil	Nil	Normal	Normal

(18)

1.7 Management

1.7.1 The following stool investigations are recommended

Detailed laboratory inspection of stool sample is the accepted diagnostic test for CDI. (1, 8). An assortment of laboratory tests has evolved. The specific test used may differ from institution to institution. The different tests that may be used are described below.

Stool microscopy, culture and sensitivity (mcs): This is the most sensitive test available; this is however less specific due to the presence of non-toxigenic *Clostridium difficile* strains. (8)

Molecular test: The PCR assay test has the high sensitivity and specificity for detecting toxin B produced by *C-difficile*. (1)

Antigen detection: This is a rapid test that detects antigens by latex agglutination or immunochromatographic assays. This test does not distinguish the type of toxin produced. (8)

Tissue cytotoxicity: This assay detects both strains (toxin A and toxin B); it has a turnaround of approximately two days and is accepted as an accurate laboratory measure (gold standard) for diagnosis of *C.difficile.* (8)

Enzyme immunoassay: This assay identifies the presence of both toxin A and toxin B or either. However because of negative toxin A, and toxin B strain causing

disease, many institutions prefer testing for toxin B. This test is rapid and results may be available within two hours. (8)

At CHBAH, they use real time PCR for toxigenic *C.difficile* (GeneXpert). This assay specifically targets toxin B and binary toxin genes as well as the tcdC gene deletion for differentiating between toxigenic *C.difficile* and NAP1/027/B1 variant.

1.7.2 Prevention

- Appropriate antibiotic use: use antibiotics only when indicated
- Proper hand washing: all health care providers should be encouraged to wash hands with soap (not alcohol scrub) between patients
- Barrier methods: patients with CDI must be isolated and contact precautions employed and visitors should be encouraged to wash hand before and after contact with patients
- Proper cleaning of all material using soap known to kill the spores
- Community educational program to teach community about CDI and measures to control it
- Inform infection control team

1.7.3 Pharmacological

New therapies have evolved for CDI but traditional antibiotics are specifically effective against C. difficile.(19, 20,21).

- Metronidazole five hundred milligram(mg) three times per day for ten days is approved and used as first line , because it is effective and cheap

- Failure to clinical response to the above within 5-7 days prompt consideration to oral vancomycin (125mg orally, thrice a week) as second line, and may be used as first line in the following cases:
    - The organism is resistant to metronidazole
    - Allergy to metronidazole
    - Pregnant and breastfeeding patients
    - In patients where colon may not be reached (diversion of colon, ileostomy and proctosigmoidectomy).In this case it should be given via enema.Intravenous administration of vancomycin is not recommended because of the limited therapeutic concentration of the gut lumen, it must be administered orally or by enema
- Dual metronidazole with vancomycin is recommended in severe and complicated CDI
- Rifaximin
- Surgical consult is mandatory in complicated CDI
- Faecal microbiota transplantation is also an option in stable patient with recurrent CDI.

1.8 Problem statement and rationale

The prevalence of *C.difficile* and risk factors associated with it has not been defined in HIV positive patients at CHBAH.

The rationale for this study is to define prevalence and risk factors associated with CDI in HIV positive patients.

1.9 Objectives

1.9.1 Primary objective

- Primarily to ascertain the prevalence of CDI in a cohort of HIV positive patients at CHBAH.

1.9.2 Secondary objective

- To evaluate the risk factors associated with acquiring CDI in HIV positive patients.

2.0 CHAPTER 2

2.1 MATERIAL AND METHODS

2.1.1 Study Design and source of data

This was a single center prospective and cross sectional study using data of HIV positive patients who presents to a general medical ward with diarrhoea. The study period was 12 months to enable the researcher to recruit the sample size stated. Patient's demographics, drug history, co-morbid disease, HIV status, HIV-viral load (VL), CD4 count, stool analysis (mcs and PCR), Full blood count (FBC), Liver function test (LFT), Urea and electrolyte (U and E) and other variables were obtained as shown in appendix A. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (Protocol number 131032).

2.1.2 Inclusion criteria

Age >18 years

HIV positive in-patients with diarrhoea (≥ 3 stool in 24 hours)

Written consent

2.1.3 Exclusion criteria

Age <18 years

Non –hospitalised patients

HIV status unknown or negative

2.2 Sample size

HIV positive patients with diarrhoea, admitted to the medical wards at CHBAH were evaluated.

Two hundred and thirty-five patients were assessed. A sample size of 200 was established to aid completion of the study within the specified period.

2.3 Data

The data source was clinical records of internal medicine patients at CHBAH and a questionnaire was used.

2.4 Variables

- CD4 counts
- Viral load
- Co-morbidities
- Anti-TB drugs
- Antibiotics
- Use of both anti-TB drugs and antibiotics concurrently
- Proton pump inhibitors
- Laboratory variables - FBC, U & E and LFT

2.5 Data management and analysis

Data were entered into a Microsoft Excel Spread shift and transferred to STATA 14 (Statacorp) for statistical analysis. Strict confidentiality of patient's records was observed. Names of patients were not used. Means and percentages were

computed for continuous and categorical variables respectively, and compared by CDI. Mann-Whitney test was used to compare continuous variables while Chi-square or Fischer's exact tests were used to compare categorical variables.The logistic regression analysis using unadjusted and adjusted odds ratio OR 95%CI) was used. The level of significance was set at p value of 0.05.

2.6 Outcomes measures and Definition

2.6.1 Primary outcome

Positive CDI is defined as positive PCR for toxigenic *C.difficile* on stool analysis.

2.6.2 Secondary outcome

Risk factors associated with *C.difficile* were measured using regression analysis (unadjusted odds ratio confidence interval and adjusted odds ratio confidence interval).

2.7 Ethical consideration

Ethical clearance to conduct the study was obtained from the Human Research Ethics Committee (Medical) of University of the Witwatersrand. Permission was also sought from the chief executive officer of CHBH. Confidentiality was respected as no patient identifiers were used in data collection, analysis and reporting.

3.0 CHAPTER 3

3.1 RESULTS

3.1 Baseline characteristics

A total of two hundred patients from CHBAH internal medicine ward were recruited in the study over a 12-month period. The baseline characteristics are shown in table 3.1. The mean age was 41.18 years ± 10.9 (SD), 106 (53%) were male and 94 (47%) were females.

Of the studied cohort 91 (45.5%) had very low CD4 counts (<200 cells/µL), 60 (30.0%) had low CD4 count (201-499cells/µL), 36 (18%) had normal CD4 count (500-2010 cells/µL) and the remaining 13 (6, 5%) CD4 counts were unknown. The viral load was detectable in 78 (39.0%) and only 12 (6%) had a lower than detectable viral load. In 110 (55%) of these patients the viral load was not measured.

The results showed that 163 (81.5%) 158 (79.0%) and 65 (34.8%) patients had an abnormal full blood count (FBC), urea and electrolyte (U&E) and liver function test (LFT) respectively.

Of the studied cohort, 156 (78%) had concurrent co-morbidities, of which TB was noted in 74 (37.0%) patients and 82 (41.0%) had other co-morbidities. Only 44 (22%) patients had no co-morbidities.

On drug related factors, 87(43.5%) patients were on antibiotics, 107 (53.5) were not on antibiotics and for 6(3%), it was not known if they were on antibiotics or not. Out of the 87 patients who were on antibiotics, 60 (69.9 %) were on common antibiotics associated with CDI and only 3 (3.5%) were on antibiotics not commonly associated

with CDI. About 24 (27.5%) patients were on antibiotics but they did not know which type of antibiotic they used.

Patients who were on anti-TB treatment numbered 74 (37.0%) and only 8 (4%) used Proton pump inhibitors.

Of the entire study population, 53 (26.5 %) were *C.difficile* positive.

Table 3.1: Baseline characteristics of the study patients

	N=200
Demographic characteristics	N (%)
Sex	
Male	106 (53.0)
Female	94 (47.0)
Age (mean±sd)	41.18±10.9
HIV related factors	
CD4 count (cells/µL)	
Very low (<200)	91 (45.5)
Low (201-499)	60 (30.0)
Normal (500-2010)	36 (18.0)
Unknown	13 (6.5)
Viral load (copies/ml)	
LDL	12 (6.0)
Detectable	78 (39.0)
Unknown	110 (55.0)
Concurrent co-morbidites	
TB (yes)	74 (37.0)
Other (yes)	82 (41.0)
None	44 (22.0)
Laboratory characteristics	
Stool analysis (yes)	200(100%)
Abnormal FBC	163 (81.5)
Abnormal U&E	158 (79.0)
Abnormal LFT	65 (34.8)
Drug related factors	
Use of antibiotics	

Yes	87 (43.5)
No	107 (53.5)
Unknown	6 (3.0)
Use of common antibiotic associated with C.difficile (Out of 87)	
Yes	60 (69.0)
No	3 (3.5)
Don’t know	24 (27.5)
Use of Anti-TB drugs (yes)	74 (37.0)
Use of PPI (yes)	8 (4.0)
CDI (yes)	53 (26.5)

3.2 Factors associated with *C-difficile* infection

There were 53(26.5%) patients with CDI. There was no statistical difference in the proportion between males and females. Gender was statistically insignificant (p=0.73), 27 (50.9%) were males and 26 (49.1%) were females, as shown in table 3.2.The value of CD4 count (cells /µL) was statistically significant (p<0.001). From this group of patients with CDI, 41 (77.4 %) had a very low CD4 count (<200 cells/µL), 9 (17.0%) had a low CD4 count (201-499 cells/ µL) only 1 (1.8%) had a normal CD4 count (500-2010 cells/µL) and 2 (3.8%) had unknown CD4 counts. Viral load level were also statistically significant (p<0.001). None from the patients whose viral load were checked had a lower than detectable viral load, 38 (71, 7%) had a detectable viral load and 15 (28.3%) of these patient's viral load was not known.

The presence of co-morbidities was statistically significant (p<0.001).Tuberculosis was noted in 36 (67.9%) and 17 (32.1%) had other co-morbidities. Antibiotic use was statistically significant (p<0.001) and the majority of these patients 41(77.4%) used antibiotics, only 10 (18.8%) did not use antibiotics. A small number, 2 (3.8%) did not know if they used antibiotics, none used uncommon antibiotics (antibiotic not commonly associated with CDI). Common antibiotic use was noted in 32 (60.4%) and only 9 (17%) did not know which antibiotic they used. There were 12(22.6%) patients who were only on anti-TB treatment and not antibiotics. Anti-TB drugs use was statistically significant (p<0.001), with 36 (67.9 %) patients that used anti-TB therapy and 17 (32.1%) did not use anti-TB therapy because they did not have TB and 27 (50.9%) used both antibiotics and anti-TB drugs.

Table 3.2: Factors associated with *c-difficile* positive

Variables	**c-diff positive (n=53)**	**p-value**
Sex		0.73
Male	27 (50.9)	
Female	26 (49.1)	
CD4 count (cells/µL)		<0.001
Very low (<200)	41 (77.4)	
Low (201-499)	9 (17.0)	
Normal (500-2010)	1 (1.8)	
Unknown	2 (3.8)	
Viral load (copies/ml)		<0.001
LDL	0 (0.0)	
Detectable	38 (71.7)	
Unknown	15 (28.3)	
Concurrent morbid conditions		<0.001
TB (yes)	36 (67.9)	
Other (yes)*	17 (32.1)	
Use of antibiotics		<0.001
Yes	41 (77.4)	
No	10 (18.8)	
Unknown	2 (3.8)	
Antibiotic type		<0.001
Uncommon	0 (0.0)	
Common	32 (60.4)	
Unknown	9 (17.0)	
Not on antibiotic but on TB treatment only	12 (22.6)	
Use of Anti-TB drugs		<0.001

Yes	36 (67.9)	
No	17 (32.1)	
Both antibiotic use and anti-TB drug use	27 (50.9)	<0.001

Legend

- Comorbidities identified: Meningitis, pneumonia, hematological and non-hematological malignancies

3.3 Comparison of CDI Negative with CDI POSITIVE

Mean age for CDI positive was higher than CDI negative (z=2.226,p=0.03), this was a continuous variable, however this had a nominal distribution.

Gender did not reveal any difference in the distribution percentage of CDI positive and CDI negative.

The percentage of CDI positive with very low CD4 count was higher than the percentage of CDI negative with very low CD4 count. (p<0.001)

The same observation was also consistent with detectable viral load in CDI positive and CDI negative. (p=0.001).

Patients with CDI and had TB had a higher percentage distribution than the patients of the same group who were CDI negative. (p<0.001)

Antibiotic use revealed a higher percentage of distribution in CDI positive than CDI negative. (p<0.001)

The use of anti-TB drugs also revealed a higher percentage of distribution compared to patients who were CDI negative on anti-TB therapy. (p<0.001)

Table 3.3: Comparison of patients who are CDI-positive with CDI-negative patients

Variable	**Patients**		
	CDI-positive (N=53)	**CDI-negative(N=147)**	**p-**

	n (%)	n (%)	value
Demographic characteristics			
Age (mean±sd)	38.28±9.0	42.23±11.3	**0.03**
Sex			0.73
Male	27 (50.9)	79 (53.7)	
Female	26 (49.1)	68 (46.3)	
HIV related factors			
CD4 count (cells/uL)	n=51	n=136	**<0.001**
Very low (<200)	41(80.3)	50(36.8)	
Low (201-499)	9(17.7)	51(37.5)	
Normal (500-2010)	1(2.0)	35(25.7)	
Viral load (copies/ml)	n=38	n=52	**0.001**
LDL	0 (0.0)	12 (23.1)	
Detectable	38 (100.0)	40 (76.9)	
Concurrent morbid conditions	**n=53**	**n=103**	**<0.001**
TB (yes)	36 (67.9)	38(36.9)	
Other (yes)	17 (32.1)	65(63.1)	
Drug related factors			
Use of antibiotics	**n=51**	**n=143**	**<0.001**
Yes	41 (80.4)	46 (32.2)	
No	10 (19.6)	97 (67.8)	
Use of Anti-TB drugs	**n=52**	**n=146**	**<0.001**
yes	37 (71.2)	37 (25.3)	

3.4 Types of antibiotics used by the study participants

Overall (87 who used antibiotics and 12 who were on anti-TB but did not use antibiotics) 60.61% of the study participants were on common antibiotics associated with CDI and 24.24% did not know the type of antibiotics they used. About 12.12% of the study participants were not on antibiotics but were on first line anti-TB treatment, and 3.03% of the study participants were on antibiotics not commonly associated with CDI.

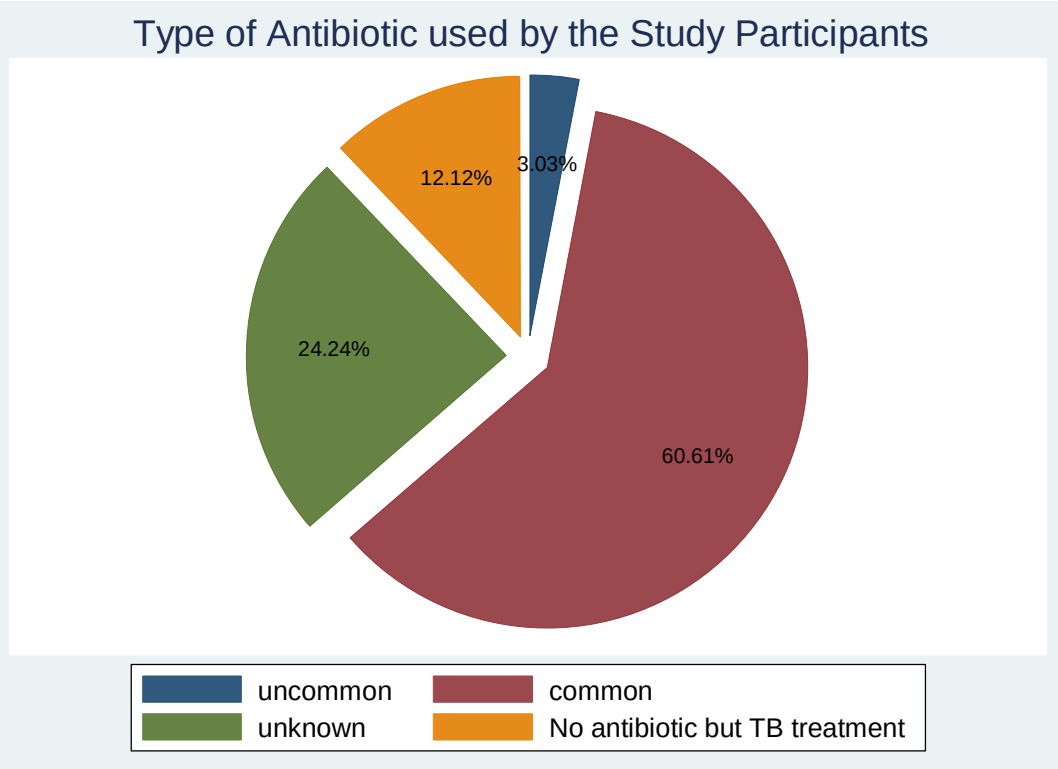


Figure 3.1: Type of antibiotics used by participants

3.5 The unadjusted and adjusted odds ratio (OR) of risk factors for *C.difficile* positive across selected variables

3.5.1 Adjusting for CD4 counts

A low CD4 count was found to be a predictor for CDI (p=0.001), after adjusting for the confounders, a low CD4 count was no longer a strong predictor (p=0.62) which was statistically insignificant. This then explains that a low CD4 count was not the only variable for CDI; other factors contributed to CDI.

3.5.2 Adjusting for viral load

Viral load levels were found to be a strong predictor for CDI (p<0.001); this was statistically significant and this was **unadjusted.**

3.5.3 Adjusting for co-morbidities (TB)

Having TB was a predictor for CDI; this was statistically significant (p<0.001) however other factors, other than TB, contributed to CDI.

3.5.4 Adjusting for antibiotic use

Using common antibiotics associated with CDI was a strong predictor for CDI (p<0.001). After adjusting for confounders, the use of antibiotics associated with CDI remained a strong predictor of outcome (CDI), thus use of common antibiotics is associated with risk of CDI.

3.5.5 Adjusting for anti-TB drugs

The use of anti-TB drugs was a strong predictor for CDI (p<0.001). After adjusting for confounders, the use of anti-TB drugs remained a strong predictor of outcome.

Table 3.4: Illustrates the unadjusted and adjusted odds ratio OR of CDI across selected variables

Variable	Unadjusted OR (95%CI)	p-value	Adjusted OR (95% CI)	p-value
CD4 count (cells/µL)				
Normal(Ref)	–	–	–	–
Low	6.18 (0.75-50.96)	0.09		
Very low	28.70 (3.77-218.57)	0.001	1.83 (0.17 – 19.55)	0.62
Viral load (copies/ml)				
LDL(Ref)	–	–	–	–
Detectable	6.02 (2.98 – 12.15)	<0.001	–	–
Concurrent morbid conditions				
Other (Ref)	–	–	–	–
TB	3.62 (1.80-7.31)	<0.001	–	–
Use of antibiotics (yes)	8.65 (3.98-18.77)	<0.001	16.80 (5.41 – 52.21)	<0.001
Use of Anti-TB drugs (yes)	8.14 (3.77-17.56)	<0.001	6.78 (2.25 – 20.43)	0.001
R-squared			0.38	

3.5.6 Regression model for using both anti-TB drugs and common antibiotics

The R-squared of 0.38 is the statistical measure of how close the data were fitted to the regression model; this explained the risk of CDI when using both anti-TB drugs and common antibiotics associated with CDI.

4.0 CHAPTER 4

4.1 Discussion

4.1.1 Prevalence of *C.difficile* infection

Clostridium difficile is one of the important organisms causing diarrhoea and, if not properly treated, it can lead to serious side effects, even death.(1) The prevalence of *C.difficile* in HIV positive patients presenting with diarrhea was found to be 53 (26.5%) out of 200 studied patients over a 12 month period.

4.2 Risk factors associated with *C.difficile*

4.2.1 *C.difficile* and CD4

Low CD4 count (201-499 cells µ/L) was not a strong predictor for CDI (p = 0.09) and unadjusted odds ratio OR (95%CI) was 6.18 (0.75-50.96), but the very low CD4 count (<200cellsµ/L) was statistically significant (p=0.001) with unadjusted odds ratio OR (95%CI) of 28.70 (3.77-21.8) but when adjusted, it was not a strong predictor for CDI (p= 0.62) as described in the results section. We can thus deduce from this study, that CD4 count was not the only risk factor associated with CDI.

4.2.2 *C.difficile* and viral load

The viral load and CD4 count are the surrogate markers for anti-retroviral treatment and disease progression and have been used to manage and monitor HIV infection for decades, thus if your HIV viral load is low you are less likely to have infection (opportunistic and non-opportunistic)(22). This was the case in this study, where patients with high viral load had risk of CDI (p<0.001), the unadjusted odds ratio OR

(95%CI) was 6.02 (2.98-12.15), the Confidence interval was not broad; however the relation between CDI and HIV viral load has not been studied. High viral load was a risk factor for CDI but other factors other than viral load level contributed to CDI

4.2.3 *C.difficile* and concurrent co-morbidities

There were 74(37.0%) patients who were infected with TB from the cohort of 200. The CDI population 53 (26.5%) with TB were more than half 36 (67.9) and having TB was a predictor for CDI (P<0.001) with unadjusted odds ratio OR and narrow (95% CI) of 3.62 (1.80-7.31). These patients were already diagnosed with TB before presenting at CHBAH. This could be attributed to the fact that TB is one of the most common opportunistic infections in HIV positive patients in the world.

A significant proportion, 82(41.0%) of the studied cohort had other co-morbidities, (i.e.) Community acquired pneumonia, meningitis, HIV associated malignancy, etc. From the CDI population, 17(32.1%) had other comorbidities. There was no direct correlation noted between these co-morbidities and the risk of CDI.

4.2.4 *C.difficile* and anti-TB drugs

The use of anti–TB drugs (all first line) was a strong predictor for CDI (p< 0.001); it remained a strong predictor after adjusting for confounders with adjusted OR and narrow therapeutic (95%CI) of 6.78 (2.25-20.43). Using anti-TB drugs was a risk factor for CDI.

4.2.5 *C.difficile* and antibiotics

The common antibiotic associated with C.difficile was found to be a single risk factor of CDI ($p<0.001$) with unadjusted and adjusted odds ratio OR (95%CI) of 8.65 (3.98-18.77) and 16.80 (5.41-52.21) respectively.

4.2.6 *C.difficile* with both anti-TB drugs and antibiotics

The R-squared after adjusting for confounders was 0.38, thus there was a 38% chance risk of having CDI if patients were using both antibiotics and anti –TB drugs.

4.3 Relations and difference of findings with literature

4.3.1 Diarrhoea and HIV

A study was done in North India where stool was sent for analysis of 200 HIV positive patients; it was found that the lower the CD4 count, the more susceptible patients were to diarrhoea and protozoa was the most identified organism; this is because of impaired immunity (23).

4.3.2 *C.difficile* and CD4 count

The effect of a low CD4 count on the risk of CDI has not been well described. A study was done at John Hopkins with a HIV clinical cohort where they assessed the incidence of CDI between 01 July 2003 and– 31 December 2010. They concluded that incidence of CDI in HIV positive patients was twice that previously reported; they ascribed this to compromised immunity defined by CD4 <50 cellsμ/L (24, 25).

This however, was not supported in our study as adjusted odds ratio showed that low CD4 count (<200cellsμ/L) was not the only variable associated with risk of CDI

(p=0.62); this was statistically insignificant. There were other risk factors associated with CDI.

4.3.3 *C. difficile* and other co-morbidities

The occurrence of CDI and HIV is established in literature (10). However the influence of CDI with other co-morbidities has not been well reviewed in literature. In South Africa, HIV is common and most of our patients, when seeking health care, they usually have more than one pathology, and especially TB associated conditions (unpublished data).

4.3.4 *C.difficile* and anti-TB drugs

There is minimal data on the effect of anti-TB drugs on CDI. A study in Poland suggested that CDI should be considered in patients who developed diarrhoea whilst on anti-TB medication, especially rifampicin.(26) We found that patients who were on anti- TB drugs were at increased risk of developing CDI an identifiable risk factor of CDI ($p<0.001$). These patients were on first line TB therapy. Some of these patients were using both anti-TB drugs and antibiotics which could influence the development of CDI (R-squared 0.38).

4.3.5 *C.difficile* and antibiotics

There is substantial evidence in literature on the risk of CDI with antibiotic use. (17,18). Our study findings were consistent with this , antibiotic use remained a strong predictor of outcome even after adjusting for confounders, with results

showing adjusted and unadjusted ratio and (95%CI) of 8.65 (3.98-18.77) and16.80 (5.41 – 52.21) respectively .

4.3.6 *C.difficile* and proton pump inhibitors

Only 8 (4%) from the 200 study participant patients were using proton pump inhibitors; the sample size was not enough to study its relation with CDI in our study, however literature has described the use of PPI as an additional risk factor to antibiotic use. This is however, still controversial (27, 28).

4.4 Limitations of this study

- Inadequate data (i.e). CD4 counts and viral loads were not available for all patients.
- Inadequate patient histories, specifically related to antibiotic usage in the period prior to admission
- Some patients were too sick to recall treatment given prior to hospitalisation.
- Some of our patients did not know the names of antibiotics given to them prior to admission
- A positive PCR is not diagnostic of CDI, it may be indicative of an asymptomatic carrier state or colonization without disease.

4.5 Conclusion

- In this study, 200 HIV positive patients presenting with diarrhoea were evaluated for the presence of CDI and the risk factors associated with it.
    - CDI was detected in 53(26.5%).
- The risk factors identified in our group were:
    - Anti- tuberculosis drug use (specifically Rifampicin)
    - Antibiotic use
    - Using both antibiotics and anti-Tuberculosis drugs
    - High viral load
    - Co-morbidities
- CD4 count was not identified as a risk factor

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6.0 APPENDICES

6.1 Appendix 1 Copy of data collection sheet

Gender:	Male	Female		
Race:	Asian	Black	Coloured	White
Age:		Unknown		
RVD:	Positive	Negative	Unknown	
CD4 count:	Value	Unknown		
Viral Load:	Value	Unknown		
Co-morbid Disease:	Yes	No		
If yes, specify:	Tuberculosis	Other:		
Diagnostic Tool:	Rapid	PCR	If none of the above, which diagnostic tool was used?	
C-diff :	Positive	Negative		
Recent antibiotic exposure:	Yes	No	If yes, which one and duration before infection?	
Use of TB drugs:	Yes	No	Unknown	
Use of both TB drugs and antibiotics:	Yes	No	Unknown	
Use of PPI:	Yes	No	Unknown	
If yes briefly state when, and duration before infection				
Clinical information and Lab Results FBC, U&E, LFT if available				
FBC	Normal	Abnormal	Unknown	
U+E	Normal	Abnormal	Unknown	
LFT	Normal	Abnormal	Unknown	

6.2 Appendix 2: Copy of ethics approval

R14/49 Dr Thulisani P Shabangu

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131032

NAME: (Principal Investigator)	Dr Thulisani P Shabangu
DEPARTMENT:	Department of Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Clostridium Difficile at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	25/10/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Reid Ally
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	25/11/2013

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