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A PROSPECTIVE STUDY OF CLOSTRIDIUM DIFFICILE INFECTION TO INVESTIGATE THE IMPACT OF THE NAP1 STRAIN IN A TERTIARY REFERRAL HOSPITAL

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I, Dr Naayil Rajabally, declare that the research reported is based on independent work; neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. This work has not been reported or published prior to registration for the abovementioned degree.
DEDICATION

To my dearest wife, Shaheen – Your ever-lasting support and selfless nature have allowed me to reach my goals. I am blessed to have you in my life and I love you infinitely.

To my boys, Aadil and Ismail – Your innocent faces and beautiful smiles keep me going.

To my parents, Abdool Mutallib and Sahidah – It is through your sacrifices that I stand where I am today. There are no words to thank you both for that.
TABLE OF CONTENTS

DEDICATION ........................................................................................................................................................ 1

ABSTRACT ........................................................................................................................................................ 5

  Background and Objectives ............................................................................................................................ 5

  Methods ........................................................................................................................................................ 5

  Results .......................................................................................................................................................... 5

  Conclusion ................................................................................................................................................ 6

ABBREVIATIONS ................................................................................................................................................. 7

PART A – PROTOCOL ......................................................................................................................................... 8

  Background ................................................................................................................................................ 9

  Study Hypothesis ...................................................................................................................................... 11

  Aims of Proposal ...................................................................................................................................... 11

  Significance of Proposal ........................................................................................................................... 12

  Patients and Methods ............................................................................................................................... 13

  Statistical Analysis ................................................................................................................................ 14

  Identification of NAP 1 ............................................................................................................................... 14

  Ethics Consideration ................................................................................................................................. 15

  Budget ....................................................................................................................................................... 15

  References ................................................................................................................................................. 16

PART B – LITERATURE REVIEW ...................................................................................................................... 17

  Objectives ............................................................................................................................................... 17

  Literature Search Strategy ........................................................................................................................ 17

  Inclusion, Exclusion and Quality criteria ................................................................................................. 17

  Introduction ............................................................................................................................................. 18

  Risk Factors ............................................................................................................................................ 19

  Epidemiology ........................................................................................................................................... 21

  Diagnosis .................................................................................................................................................. 23

  Laboratory Tests ...................................................................................................................................... 24

  Molecular characterisation ....................................................................................................................... 25
Illustrations and Tables................................................................. 55
References ....................................................................................... 56
Proofs ............................................................................................... 57
Change of Address .......................................................................... 58
CPD Points ..................................................................................... 58
Charges ............................................................................................ 58
Ethics Approval Letter ...................................................................... 59
Author Contributions to Study ....................................................... 61
Acknowledgements ........................................................................... 61
ABSTRACT

BACKGROUND AND OBJECTIVES

The aim of this study is to report the incidence of *Clostridium difficile*-associated disease (CDAD) in a tertiary care hospital in South Africa and to identify risk factors, assess patient outcomes and determine the impact of the hypervirulent strain of the organism referred to as North American pulsed-field type 1 (NAP1).

METHODS

Adults who presented with diarrhoea over a period of 15 months were prospectively evaluated for CDAD using stool toxin enzyme immunoassay (EIA). Positive specimens were evaluated by PCR. Patient demographics, laboratory parameters and outcomes were analysed.

RESULTS

CDAD was diagnosed in 59 (9.2%) of 643 patients (median age 39 years, IQR 30 - 55). Thirty-four (58%) were female. Recent antibiotic exposure was reported in 39 (66%), 27 (46%) had been hospitalised within 3 months, and 14 (24%) had concomitant inflammatory bowel disease (IBD). Nineteen (32%) had community-acquired CDAD (CA-CDAD). The annual incidence of hospital-acquired CDAD (HA-CDAD) was 8.7 cases/10 000 hospitalisations. Two cases of the hypervirulent strain NAP1 were identified. Seven (12%) patients underwent colectomy (OR 6.83; 95% CI 2.41 - 19.3). On logistic regression, only antibiotic exposure independently predicted for CDAD (OR 2.9; 95% CI 1.6 - 5.1). Three (16%) cases of CA-CDAD reported antibiotic exposure (v. 90% of HA-CDAD, p<0.0001). Twelve (86%) patients had concomitant IBD (p<0.0001 v. HA-CDAD). CA-CDAD was significantly associated with antibiotic exposure (OR 0.04, 95% CI 0.01 - 0.24) and IBD (OR 9.6, 95% CI 1.15 - 79.8).
CONCLUSION

The incidence of HA-CDAD in the South African setting is far lower than that reported in the West. While antibiotic use was a major risk factor for HA-CDAD, CA-CDAD was not associated with antibiotic therapy. Concurrent IBD was a predictor of CA-CDAD.
ABBREVIATIONS

CDAD - *Clostridium difficile*-associated disease

CA-CDAD - community-acquired *Clostridium difficile*-associated disease

CDI - *Clostridium difficile* infection (CDI)

HA-CDAD - hospital-acquired *Clostridium difficile*-associated disease

IBD - Inflammatory Bowel Disease

NAP 1 – North American pulsed-field type 1

PMC - Pseudomembranous colitis

PPI - Proton Pump Inhibitors
PART A – PROTOCOL

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UCT REC REF: 310/2008
BACKGROUND

*Clostridium difficile* (*C. difficile*) was originally identified as the cause of antibiotic-associated pseudomembranous colitis (PMC) in 1978.\(^1\) Exposure to antimicrobials, such as clindamycin or ciprofloxacin, alters the normal bowel flora and promotes *C. difficile* overgrowth with the subsequent production of toxins A and B. These toxins can induce intense inflammation of the colonic mucosa resulting in severe diarrhoea, fever, abdominal pain and leucocytosis. *C. difficile*-associated diarrhoea (CDAD) presents with a spectrum of clinical manifestations ranging from mild symptoms to life threatening colitis.\(^2\),\(^3\),\(^4\),\(^5\),\(^6\) CDAD typically affects the elderly, either hospitalised patients or long-term care facility residents. The ability of *C. difficile* to form spores is thought to be a key feature in enabling it to persist in patients and in the physical environment for prolonged periods, thereby facilitating its transmission. The causative organism is acquired by the oral route from an environmental source or by contact with an infected person or a health care worker who serves as a vector. CDAD is associated with increased health-care costs, prolonged hospitalisations and increased patient morbidity.

Since 2000, there has been renewed interest in CDAD following a dramatic increase in both incidence and severity reported from the West, initially from Quebec.\(^7\),\(^8\),\(^9\),\(^10\),\(^11\) Data from the US Centre for Disease Control and Prevention (CDC) reveal that hospitalisations with a discharge diagnosis of CDAD have significantly increased from 31 per 100,000 population in 1996 to 61 per 100,000 in 2003.\(^11\) This doubling of cases has also been associated with increased patient mortality. This striking change in epidemiology, clinical severity and case-fatality ratio is attributable to the emergence of a strain that has been identified as the North American pulsed-field type 1 (NAP1). The hypervirulent NAP1 strain produces increased amounts of Toxins A and B as well as an additional toxin named the binary toxin. NAP1 has been responsible for increased morbidity and mortality among hospitalised patients and in
particular patients with Inflammatory Bowel Disease (IBD). There are also reports of severe
disease in otherwise healthy persons in the community – community-associated CDAD (CA-
CDAD). The “Quebec experience” reported an attributable mortality rate of 17%, with >1400
deaths and a recurrence rate in patients >65 years of 58%.

Preliminary data from a retrospective review of CDAD at Groote Schuur Hospital reveals an
almost 2 fold increase in the incidence of the infection in both IBD and non-IBD cohorts over
the past 4 years. We hypothesise that this is suggestive of a potential new strain in the local
setting.

Early typing techniques were based on phenotypic characteristics such as antibiotic resistance
patterns, soluble protein patterns, bacteriophage and bacteriocin patterns, and Western
immunoblotting. Genotype-based methods such as restriction endonuclease analysis of the
total bacterial genome, pulsed-field gel electrophoresis, and primed PCR were introduced in
the 1980’s. There are several techniques employed to distinguish NAP1 from other strains.
Killgore et al provided a comprehensive comparison of seven techniques for typing
International Epidemic strains of *C. difficile*; Restriction Endonuclease Analysis, Pulsed-
Field Gel Electrophoresis, PCR-Ribotyping, Multilocus Sequence Typing, Multilocus
Variable-Number Tandem Repeat Analysis, Amplified Fragment Length Polymorphism, and
Surface Layer Protein A Gene Sequence Typing. Their conclusion was that, most of the
above techniques were able to cluster isolates that appeared to be related, and PCR-ribotyping
results had very good inter-laboratory agreement.

Traditionally, the treatment of CDAD is to discontinue the implicated agent, and in those
with moderate or severe disease, to administer oral metronidazole or vancomycin. Oral
vancomycin is the preferred drug for patients who are seriously ill or who fail to respond
rapidly to metronidazole. With the emergence of NAP1 strain, the treatment of *C. difficile
infections has become more challenging in that the disease is more severe in nature with significant complications such as severe colitis, paralytic ileus and toxic megacolon. In addition, it is more refractory to standard treatment. Hence, new therapies geared at targeting this new strain will become vital in the near future.\textsuperscript{14}

**STUDY HYPOTHESIS**

We postulate a positive relationship between the occurrence of the NAP1 strain and the increasing incidence of \textit{C. difficile} infection recently observed in our institution.

**AIMS OF PROPOSAL**

The aim of this study is to prospectively identify the magnitude of CDAD (both community and hospital acquired) attributable to the NAP1 strain in our referral Health Care facility and to evaluate the impact of the strain on patient morbidity and mortality.

In order to achieve our aims, we propose:

1. To develop a cost-effective and practical method to identify the NAP1 strain using PCR in Professor Pretorius’ laboratory at Pathcare
2. To capture data on CDAD symptoms, markers of severity, length of hospital stay, associated antibiotic use, co-morbid illnesses, recurrence rate, complications, the need for surgery and mortality associated with the NAP1 strain.

The following CDC recommended definitions will be used to determine study endpoints\textsuperscript{15}:

- CDAD will be defined as diarrhea and a stool sample positive for \textit{C. difficile} toxin A, as determined using an enzyme immunoassay (EIA), or diarrhoea and typical features of PMC (regardless of toxin EIA results).
- A complicated course of CDAD will be defined as admission to an intensive care unit, surgical intervention, or death associated with CDAD.
A case will be considered to have been nosocomially acquired if the diarrhoea started >48 hours after admission to the hospital, if the patient resides in a long-term care facility or was discharged from a hospital or long-term care facility within 14 days before onset.

Community-onset CDAD will be defined as diarrhea that started before hospital admission or within 48 hours of admission.

Recurrence will be defined as an episode that occurred <8 weeks after the onset of a previous episode.

Severe diarrhoea will be defined as bloody diarrhoea or diarrhoea with one or more of the following: hypovolemia, fever, leukocytosis, hypoalbuminemia or pseudomembranous colitis.

Mortality will be attributable to CDAD if a patient dies of the consequences of CDAD during hospitalisation or within 30 days of discharge.

SIGNIFICANCE OF PROPOSAL

CDAD has emerged to become an important and widespread health problem. With the extensive use of antimicrobials and an ageing population with improving access to healthcare, CDAD has the potential to rise to an alarming proportion.

The NAP1 strain has proven to be the single most important epidemic strain causing CDAD. In countries such as the United States, Canada and the United Kingdom the above-mentioned strain has caused severe disease, even in healthy persons in the community, so much so that the State of Connecticut in the United States has added CA-CDAD to the list of reportable conditions in January 2006. In South Africa, data suggest that CDAD is on the increase. However, we do not have an established technique to detect this virulent strain, and we do not have data on the morbidity and mortality associated with the latter. Thus, it will be of great value to devise a study in order to identify and gauge the impact of the NAP1 strain of C.
difficile in the local healthcare setting. Should our hypothesis prove correct, this will likely have enormous impact on our hospitals antibiotic policy. Furthermore, identification of this virulent strain will provoke the need to establish CDAD as a notifiable condition in our country.

PATIENTS AND METHODS

All adult patients admitted to Groote Schuur Hospital from the community with diarrhoea (defined as ≥ 3 watery bowel actions per day for more than 24 hours) or who develop diarrhoea following admission for an unrelated condition, will be included in the study cohort. The study will be conducted over a 6 month period.

Appropriate patients will be identified from daily visits by study investigators to all hospital wards, intensive care units or following referral from medical colleagues. All Groote Schuur Hospital medical practitioners will also be notified of the study via email with clear instructions on who to enrol. Posters will also be displayed in both medical and surgical wards. In addition, investigators will be alerted by the Department of Microbiology on receipt of diarrhoeal stool from any Groote Schuur inpatient facility.

Inpatients attending the gastrointestinal clinic with endoscopic features of pseudomembranous colitis will also be recruited into the study. These patients will be undergoing endoscopy at the discretion of their attending clinician for standard clinical indications. Endoscopy will not be undertaken as part of this study.

In all cases, a fresh stool specimen will be tested by Microbiology for the presence of C. difficile Toxin A using standard EIA testing. More than one stool sample may be tested, as clinically indicated (should the first test negative for C. difficile in subjects in whom there is a high index of clinical suspicion).
All samples testing positive will then be dispatched to PathCare laboratory for NAP1 strain typing. The technique is described below.

All positive results will be reported to the attending clinician to ensure standard therapy with either metronidazole or oral vancomycin is given as soon as possible.

All patients will be interviewed with respect to patient demographics, recent antibiotic use, previous hospitalisation and comorbid illnesses. Data pertaining to laboratory tests will be extracted from inpatient hospital records. These data will be stored on a confidential database, which only the principal investigators will have access to, ensuring that patient confidentiality is maintained.

STATISTICAL ANALYSIS

Statistical analysis will be performed using Stata Statistical Software: Release 11 (StataCorp, College Station, USA). Continuous variables are to be expressed as medians and interquartile ranges. The Mann-Whitney test will be used to assess continuous variables, while the chi-square or Fisher's exact test will be used for categorical variables. Univariate analysis will be performed initially for each variable. Variables differing between groups with a significance level of $p<0.1$ and other possible confounders identified a priori, will then be entered into a series of multivariate logistic regression models.

IDENTIFICATION OF NAP1

DNA will be extracted from stool samples using the Roche Magnapure automated extraction robot. Extracted DNA will then be subjected to 2 rounds of real-time PCR. The first round will be to confirm the presence of the two toxin genes, according to Belanger et al.17 This assay makes use of molecular beacon probes specific for the 2 genes, helping to distinguish between $\text{A}^+\text{B}^+$ and $\text{A}^-\text{B}^+$ strains. It will be done on the Corbett Rotor-Gene 6000 platform. The second real-time PCR is to identify NAP1 isolates as described by Sloan et al by
amplifying the tcdC gene, which is a control gene for the two toxin genes. A deletion of 18 base pairs in this gene leads to higher levels of toxin expression, hence the increased virulence of these isolates. The deletion can be identified by using hybridization probes on the Roche Lightcycler instrument, where a shift in denaturation temperature denotes the presence of the deletion.

ETHICS CONSIDERATION

This study protocol has been approved by the Ethics committee, University of Cape Town.

BUDGET

- C difficile EIA toxin testing R 15 000
- Molecular Analysis: general chemicals, gloves etc
- DNA extraction, Gels R 25 000
- Nucleotide Polymorphism analysis R 40 000
- Data collector/Interviewer R 10 000
- Transportation of stools to Pathcare R 5 000

Total R 95 000
5. Wilcox MH. Cleaning up Clostridium difficile infection. Lancet 1996; 348:767
10. McDonald LC. Clostridium difficile: responding to a new threat from an old enemy. Infect Control Hosp Epidemiol 2005; 26:672-67
PART B – LITERATURE REVIEW

OBJECTIVES

The purpose of the literature review is to investigate the incidence of *C. difficile*-associated disease (CDAD) as reported in other countries and to compare this to local experience, with a view to gauging the magnitude of the disease and identifying the need for any further research on the topic. Further investigating the epidemiology of CDAD will allow for the identification of potential new strains, type of disease acquisition – community or hospital-acquired, recognition of major risk factors, evaluation of patients’ clinical outcome and assessment of disease severity. By defining *Clostridium difficile* (*C. difficile*) and examining its virulence factors, disease pathogenesis will be discussed. The recommended diagnostic modalities for *C. difficile* will be evaluated and compared with the existing strategy at our institution. Lastly, the treatment of CDAD will be reviewed with a focus on the potential need for more aggressive treatment in severe cases.

LITERATURE SEARCH STRATEGY

Computerized English-language literature searches of PubMed were performed in order to yield robust peer-reviewed articles on the subject matter. The following key terms were used – “Clostridium difficile,” “antibiotic-associated diarrhoea,” “hospital-acquired diarrhoea”, “pseudomembranous colitis” and “NAP1”. In addition, similar searches were performed on Google Scholar as it also provided pertinent non-peer-reviewed materials.

INCLUSION, EXCLUSION AND QUALITY CRITERIA

Various criteria were set out to decide which materials to use and which were to be excluded; this exercise was performed in order to ensure that high-quality research reports and articles are included in the review. The criteria are summarised below.
1. No date restriction was applied in the literature search so as to gain a historical perspective as well as epidemiological insight on *C. difficile*.

2. No geographical restrictions were applied.

3. Only studies dealing with *C. difficile* in humans were selected; studies in children were excluded.

4. Materials that contributed to a comprehensive understanding and critical interpretation of the existing literature were selected.

5. Where articles discussed similar topics, the one in a more renowned peer-reviewed journal was usually favoured. However, non-peer-reviewed material was not automatically dismissed on that basis.

6. Where studies investigated similar issues, the most recent study was selected to provide a most up-to-date review.

**INTRODUCTION**

*Clostridium difficile* (*C. difficile*) is a Gram positive spore-forming anaerobic bacillus that causes disease with a wide spectrum of severity, ranging from asymptomatic colonisation to severe diarrhoea, pseudomembranous colitis (PMC), toxic megacolon, colonic perforation and death in humans (see Appendices 1.1 and 1.2).\(^{19}\) *C. difficile* was first described by Hall and O’Toole in 1935 as a component of the normal intestinal flora of newborn infants.\(^{20}\) In the 1970’s, it became increasingly recognised as an important cause of pseudomembranous colitis, particularly in patients who were receiving clindamycin.\(^{21}\) It is now the most common cause of infectious diarrhoea in the healthcare setting and accounts for 15-25% of all antibiotic-associated diarrhoea.\(^{22}\)

After colonisation, *C. difficile* causes disease by producing two highly potent exotoxins, Toxins A and B, which share amino acid homology and are considered to be the main
virulence factors of the organism.\textsuperscript{23, 24} Strains that do not produce toxins are non-pathogenic. These toxins are monoglucosyltransferases that modify rho proteins in host cells leading to collapse of the actin cytoskeleton and cell death.\textsuperscript{25} Toxins A and B are transcribed from a pathogenicity locus that comprises five genes: two toxin genes, tcdA (toxin A) and tcdB (toxin B), and three regulatory genes, one of which – tcdC – encodes a putative negative regulator of toxin transcription.\textsuperscript{23, 24, 26} It has been noted that strains carrying mutations of the tcdC area prevent the formation of the TcdC protein which would normally halt toxin production.\textsuperscript{26, 27} Such strains, therefore, are hypervirulent since their toxin production capacity is increased by 10-20 folds.\textsuperscript{26} In addition, it has also been found that hypervirulent strains produce an additional toxin called the binary toxin. Although the latter’s role is unknown it is thought to act synergistically with toxins A and B in mediating disease.\textsuperscript{23, 24, 26}

**RISK FACTORS**

Most studies evaluating risk factors have shown that exposure to an antimicrobial agent is one of the most important risk factors for the development of *C. difficile*-associated diarrhoea (CDAD).\textsuperscript{28} Antimicrobials suppress the normal gut flora, thereby, allowing *C. difficile* to flourish and colonise an appropriate host. Johnson et al reported on a highly resistant strain responsible for outbreaks in hospitals where clindamycin use was frequent; they concluded that clindamycin exposure was a specific risk factor for CDAD.\textsuperscript{29} Pepin and colleagues further reported that exposure to virtually any antibiotic, even metronidazole which is commonly used to treat CDAD, can precipitate disease.\textsuperscript{30} Numerous antibiotics exposure, more doses administered and longer exposure duration have all been associated with increased risk of CDAD.\textsuperscript{24}

Elderly patients, previous or current hospitalisation and patients requiring tube-feeding are also at increased risk. According to Pepin et al, disease rates in patients \( \geq 65 \) years of age are
10-fold higher than those for younger patients.\textsuperscript{30} \textit{C. difficile} spores are more prevalent and rapidly disseminated in certain environments such as hospitals and long-term care facilities, thereby, putting their residents at increased risk of CDAD.\textsuperscript{31,32} Spores are also resistant to alcohol-based sanitisers and ammonia-based detergents commonly used as hospital and laboratory disinfectants.\textsuperscript{33} Tube feeding and patients who require percutaneous endoscopic gastrostomy (PEG) insertion for feeding are prone to CDAD because, in addition to the potential contamination of their nutrition access by spores in the hospital environment, they are generally sicker patients requiring antibiotics.\textsuperscript{34,35}

Patients with inflammatory bowel disease (IBD), in particular those with ulcerative colitis, have been reported to be at increased risk of CDAD.\textsuperscript{36} Importantly, a higher mortality rate, longer hospital stays and increased hospital costs have been reported in patients with CDAD and concomitant IBD.\textsuperscript{36,37} Bossuyt and co-workers reported a four-fold increase of \textit{C. difficile} in patients with IBD.\textsuperscript{38} Although Nguyen postulated that immunomodulator use may partly explain the increased incidence of \textit{C. difficile} in IBD, the Bossuyt study did not support this explanation. The continued use of immunomodulators in the setting of CDAD in IBD patients remains contentious.

Out of all risk factors for CDAD, the role of gastric acid suppression seems to be the most controversial. It has been suggested that the survival and germination of \textit{C. difficile} spores are greater in the gastric contents of PPI-treated patients.\textsuperscript{39} Germination in a stomach at a higher pH environment could facilitate colonisation in a susceptible host and promote disease. Dial and colleagues found an almost four times increased risk of acquiring CDAD with concurrent PPI use, and so did Ji Won Kim et al in their retrospective analysis of risk factors for CDAD recurrence, and its relationship with proton pump inhibitors.\textsuperscript{40,41} However, in their retrospective study of 126 elderly patients, Shah et al did not find an increased risk of \textit{C.}}
difficile in those who had received PPI or H2-receptor antagonist. In addition, a recent metanalysis found very low quality evidence in support of an association between PPI use and risk of CDAD.

EPIDEMIOLOGY

Over the last decade, the epidemiology of C. difficile has been the subject of several studies predominantly coming from the West with growing concern regarding the rising incidence, disease severity and mortality associated with CDAD. The number of reported cases in the United Kingdom rose from 7470 in 1994 to 43682 in 2004, with the most devastating hospital outbreak reported at Stoke Mandeville Hospital resulting in 38 deaths in two outbreaks between 2003 and 2005. Similarly, Pepin and colleagues reported a four-fold increase in the overall incidence of CDAD in an extensive retrospective analysis from 1991 to 2003. In 2003, they described a major epidemic of C. difficile with a changing pattern of disease severity in the population studied and an overall 30-day mortality of 13.8% in that year. The most notable impact of the epidemic was in patients ≥65 years where the disease was more complicated with higher mortality.

Furthermore, the epidemiology of C. difficile has expanded in that CDAD is occurring more frequently in populations previously considered low-risk, such as healthy individuals from the community and peri-partum women in the absence of traditional risk factors as mentioned above. This expansion in the epidemiology of C. difficile has been attributed to the BI/NAP1/027 strain which carries an 18-basepair deletion mutation in the tcdC gene allowing it to produce more than 10 times the amount of toxins than conventional strains. In addition, the NAP1 strain exhibit fluoroquinolone resistance, allowing it to thrive in an environment where this particular antibiotic use is widespread. Similarly, in the Netherlands, Goorhuis et al identified a hypervirulent strain named ribotype 078 which
carries a 39–base pair deletion in tcdC, as well as a point mutation resulting in a stop codon. Although this emerging strain shared similar virulence characteristics with the NAP1 strain, it was found to affect younger patients and was more responsible for community-acquired rather than hospital-acquired disease.

In Southern Africa, data regarding \textit{C. difficile} has been scarce. Samie et al studied PCR detection of \textit{C. difficile} in adults attending an outpatient department as well as school-going children in the Vhembe district, South Africa. They found a prevalence of 14% of \textit{C. difficile} amongst their 322 study participants. About half of them were infected with toxigenic strains. The large majority of the strains were toxin A producers only. The PCR method used in their study was not designed to detect the 18-basepair deletion to identify NAP1. However, a quarter of the \textit{C. difficile} strains detected were positive for the binary toxin gene which had been expressed in NAP1 suggesting that the latter may be prevalent locally. In addition, the study did not differentiate hospital-acquired CDAD (HA-CDAD) from community-acquired CDAD (CA-CDAD) and patients’ clinical outcome had not been evaluated.

The incidence of \textit{C. difficile} in South African hospitals is yet to be determined. Lekalakala et al reported on an increase in \textit{C. difficile} at their institution in Pretoria in 2008. They reviewed their data following an increase in specimen request for \textit{C. difficile} and found a sudden increase in toxin positive samples. These were predominantly from their intensive care wards raising the suspicion of a possible outbreak. Whether or not this increase was caused by NAP1 had not been evaluated as they relied on a commercial enzyme immunoassay test detecting Toxin A only for the diagnosis of \textit{C. difficile}. It is also likely that they have missed strains that solely produce toxin B. The authors emphasized the importance
of close coordination between the laboratory, infection control teams and clinical staff in order to identify and avert possible outbreaks.

**DIAGNOSIS**

The diagnosis of CDAD is made by using a combination of clinical and laboratory criteria. The clinical presentation varies according to disease severity. The typical clinical manifestations include profuse, watery, greenish loose stools with an offensive smell associated with cramping abdominal pain in an individual who has recently received antibiotics.\(^{51}\) As the disease progresses to colitis, systemic symptoms including fever, tachycardia and leucocytosis become manifest. Overt rectal bleeding is uncommon and should prompt investigations for an alternative diagnosis. Toxic megacolon characterises fulminant colitis with marked tenderness on abdominal examination. Perforation with peritonitis should be suspected if there is rebound tenderness.

Sigmoidoscopy or colonoscopy can identify patients with pseudomembranous colitis by the visualisation of pseudomembranes which are typical yellowish-white adherent plaques on an inflamed but intact colonic mucosa without ulceration. These are pathognomonic of CDAD. Pseudomembranous colitis can occur in conditions other than CDAD, including intestinal obstruction, colon cancer, leukemia, severe burns, shock, uremia, heavy metal poisoning, hemolytic-uremic syndrome, Crohn’s disease, shigellosis, neonatal necrotizing enterocolitis, ischemic colitis, and Hirschsprung disease. However, such conditions would present with a typical set of clinical symptoms and signs. In addition, there is no colonic ulceration in CDAD as opposed to the endoscopic findings of the conditions mentioned above. Pseudomembranous colitis is a histological term used to describe summit or volcano lesions (see Appendix 1.3), depicting epithelial necrosis, goblet cells distension with mucus, oedema, and infiltration of the lamina propria with leukocytes, epithelial cells, fibrin, and mucin.\(^{51}\)
Although endoscopy provides a rapid and accurate way of making a diagnosis in a sick patient, pseudomembranes are only visible in about half of the patients with CDAD, usually when disease is advanced.\textsuperscript{51,52} In addition, endoscopy is costly, not readily available and carries a small risk of perforation in cases of paralytic ileus or toxic megacolon.\textsuperscript{53}

### LABORATORY TESTS

Several diagnostic modalities have been used to detect either the organism or its toxins, each with its own pros and cons. These include stool culture, toxigenic culture, enzyme immunoassay (EIA) for toxins A, B or both, glutamate dehydrogenase (GDH) and PCR for toxin genes. Stool culture is highly sensitive but is time-consuming and requires confirmation of toxigenic isolates by another method since it does not distinguish toxin-producing from non-toxin producing strains.\textsuperscript{54} The reliability of culture results also depends on the quality of media used.\textsuperscript{55} Its use is mainly reserved in epidemiological studies. Toxigenic culture has long been considered as the gold standard diagnostic test for \textit{C. difficile}.\textsuperscript{56,57} It is the standard against which other tests are compared.\textsuperscript{54,58} Stool is treated with heat or alcohol and then cultured on a selective agar; suspicious cultures are then identified and tested for toxin production. However, this testing strategy is cumbersome, expensive and time consuming with a turn-around time of 72-96 hours which is not useful when addressing patient care. It also requires a well-developed infrastructure for the maintenance of cell cultures which is not feasible by most laboratories.

Enzyme immunoassays (EIA) detecting either toxin A or toxin B or both are widely available at low cost and provide results within hours. However, the sensitivity of EIA tests has been reported to vary widely ranging from 30-90\%.\textsuperscript{57,59,60} EIA also performed poorly in low prevalence settings.\textsuperscript{61} Repeating the test further decreases the positive predictive value (PPV). Nevertheless, it will continue to play an important role in \textit{C. difficile} diagnosis for practical
purposes especially in resource-limited settings. Glutamate dehydrogenase (GDH) is an antigen that is present in all *C. difficile* organisms in high amounts.\(^6\) Although it is not useful as a stand-alone test, it has been shown to be a good screening test when combined with toxin EIA or a PCR-based test.\(^6\)

**MOLECULAR CHARACTERISATION**

*C. difficile* can be genotyped by several methods. Killgore et al provided a comprehensive comparison of seven techniques for typing International Epidemic strains of *C. difficile* – Restriction Endonuclease Analysis, Pulsed-Field Gel Electrophoresis, PCR-Ribotyping, Multilocus Sequence Typing, Multilocus Variable-Number Tandem Repeat Analysis, Amplified Fragment Length Polymorphism, and Surface Layer Protein A Gene Sequence Typing.\(^6\) Although this was a small study analysing 42 isolates from across Canada, USA, UK and Netherlands, the techniques described were able to cluster isolates that appeared to be related. PCR-ribotyping required relatively less labour once the reactions were optimised and the results had very good inter-laboratory agreement. PCR-based tests have consistently shown higher sensitivity and specificity when compared to EIA and GDH tests.\(^5,6\) A number of commercially available PCR-based tests have since been developed in order to provide rapid and reliable results with identification of the NAP1 strain.\(^6,6\) However, cost is a major limiting factor.

**TREATMENT**

The recommended treatment strategy for CDAD is to discontinue the inciting antimicrobial agent in the first place and to initiate treatment with either metronidazole or vancomycin depending on the severity of disease. The drug of choice is metronidazole for mild to moderate disease and oral vancomycin for more severe disease.\(^6\) Metronidazole is used as first line treatment because it is cheaper and avoidance of vancomycin would prevent the
emergence of vancomycin-resistant enterococci. Zar et al conducted a prospective, randomised, placebo-controlled trial comparing metronidazole 250 mg four times daily versus vancomycin 125 mg four times daily in 172 patients stratified according to the severity of CDAD and showed similar efficacy of the two drugs in mild disease but confirmed superiority of vancomycin for severe disease. In addition, in a retrospective study, Wilcox and Howe reported that although response and relapse rates were similar between metronidazole and vancomycin, the time to resolution of diarrhoea was significantly shorter in the vancomycin group.

With the emergence of NAP1, the treatment of C. difficile has become more challenging in that the disease is more severe and refractory to conventional medical treatment. Pepin and colleagues showed that the risk of relapse with metronidazole had increased from 9.6% in 1991-2002 to 25.7% in 2003. This may be partly explained by the epidemic in that year but it raises the possibility of development of resistance of C. difficile to metronidazole. It has been recommended to treat recurrent disease after an initial course of vancomycin with a tapering or pulse course of the latter.

Newer antimicrobial agents have been developed for the treatment of CDAD. Musher et al demonstrated efficacy of nitazoxanide in a prospective study comparing it to metronidazole. Similarly, Louie et al demonstrated that fidaxomicin was superior to vancomycin in preventing recurrences in a prospective, randomised control trial. The finding from this trial displaced vancomycin as the only drug approved by the FDA for the treatment of CDAD in 2011. Johnson et al reported cure in seven out of eight cases treated with oral rifaximin, a poorly absorbable macrocyclic antibiotic with broad spectrum antimicrobial activity against gram-negative and gram-positive organisms.
Other treatment methods have been shown to be successful in addressing the problem of recurrence. Faecal bacteriotherapy or faecal microbiota transplantation (FMT) replenishes the gastrointestinal flora and has been shown to be promising in several case studies. In a UK series, Macconnachie and colleagues established cure in 11 of 15 patients treated with FMT without any reported adverse events. A recent open-label, randomized, controlled trial performed by Van Nood et al was stopped prematurely when an interim analysis revealed a marked resolution of CDAD after the first duodenal infusion of donor faeces when compared to oral vancomycin alone or oral vancomycin followed by bowel lavage. Importantly, the patients who underwent bacteriotherapy had faecal bacterial diversity similar to the donors.

In severe cases a combination of intravenous metronidazole and oral or rectal vancomycin may be used in higher doses. Intravenous immunoglobulins have been tried in some cases with some success. But timely colectomy (see Appendix 1.4) may be the ultimate lifesaving treatment modality if there is no response to the combination treatment in cases of pseudomembranous colitis and toxic megacolon.

**RESEARCH GAPS**

The prevalence of CDAD – HA-CDAD and CA-CDAD remains unknown in South Africa. Epidemiological studies are needed to establish the burden of disease locally, to assess clinical outcomes and to determine whether NAP1 has reached epidemic proportions. Other potential hypervirulent strains in our population need to be studied.

The diagnostic modality used for *C. difficile* in most public institutions in South Africa, including ours, is toxin EIA. The performance of this test has not been assessed when compared to other available tests for *C. difficile*. 
Metronidazole is widely used as first line treatment for CDAD. The local resistance pattern to metronidazole is unknown. Should this be high enough, a change in practice may be warranted.
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The *Clostridium difficile* problem: A South African tertiary institution’s prospective perspective

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Background and objectives. The aim of this study is to report the incidence of *Clostridium difficile*-associated disease (CDAD) in a tertiary care hospital in South Africa and to identify risk factors, assess patient outcomes and determine the impact of the hypervirulent strain of the organism referred to as North American pulsed-field type 1 (NAP1).

Methods. Adults who presented with diarrhoea over a period of 15 months were prospectively evaluated for CDAD using stool toxin enzyme immunoassay (EIA). Positive specimens were evaluated by PCR. Patient demographics, laboratory parameters and outcomes were analysed.

Results. CDAD was diagnosed in 59 (9.2%) of 643 patients (median age 39 years, IQR 30 - 55). Thirty-four (58%) were female. Recent antibiotic exposure was reported in 39 (66%), 27 (46%) had been hospitalised within 3 months, and 14 (24%) had concomitant inflammatory bowel disease (IBD). Nineteen (32%) had community-acquired CDAD (CA-CDAD). The annual incidence of hospital-acquired CDAD (HA-CDAD) was 8.7 cases/10 000 hospitalisations. Two cases of the hypervirulent strain NAP1 were identified. Seven (12%) patients underwent colectomy (OR 6.83; 95% CI 2.41 - 19.3). On logistic regression, only antibiotic exposure independently predicted for CDAD (OR 2.9; 95% CI 1.6 - 5.1). Three (16%) cases of CA-CDAD reported antibiotic exposure (v. 90% of HA-CDAD, p<0.0001). Twelve (86%) patients had concomitant IBD (p<0.0001 v. HA-CDAD). CA-CDAD was significantly associated with antibiotic exposure (OR 0.04, 95% CI 0.01 - 0.24) and IBD (OR 9.6, 95% CI 1.15 - 79.8).

Conclusion. The incidence of HA-CDAD in the South African setting is far lower than that reported in the West. While antibiotic use was a major risk factor for HA-CDAD, CA-CDAD was not associated with antibiotic therapy. Concurrent IBD was a predictor of CA-CDAD.
INTRODUCTION

*Clostridium difficile* is a Gram-positive, spore-forming anaerobic bacillus that causes disease by producing cytotoxins. The clinical outcome depends on host immunity and the virulence of the toxin-producing strain.\(^1\) *C. difficile* causes disease that ranges in severity from asymptomatic colonisation to severe diarrhoea, pseudomembranous colitis (PMC), toxic megacolon, colonic perforation and death.\(^2\)

Over the last decade, there has been a dramatic increase in the incidence and severity of *C. difficile*-associated diarrhoea (CDAD), predominantly affecting western countries. The striking change in epidemiology, clinical severity and case-fatality ratio is attributable in part to the emergence of a strain, identified as the North American pulsed-field type 1 (NAP1), referred to more commonly in Europe as Type 027. This hypervirulent strain produces increased amounts of toxins and is resistant to the fluoroquinolones.\(^3\)

Data regarding the burden of CDAD in southern Africa are limited. The magnitude of *C. difficile* infection in South African hospitals is not known and it is also unclear what proportion of CDAD is community-acquired (CA-CDAD). Therefore, the aims of this study were to investigate the incidence of CDAD in a tertiary referral hospital, to evaluate associated risk factors and effect on patient morbidity and mortality, to report on the presence of NAP1 and to determine the percentage of CA-CDAD.

METHODS

The study was conducted at Groote Schuur Hospital (GSH), a 943-bed tertiary referral hospital in Cape Town. All adult patients admitted with diarrhoea, or who developed diarrhoea following admission for an unrelated condition, were evaluated prospectively over 15 months. Stool was tested for Toxin A using standard enzyme immunoassay (EIA). DNA was extracted from toxin-positive stool and subsequently subjected to real-time PCR.
Demonstration of an 18-base pair deletion of the tcd C gene, after amplification, indicated the presence of NAP1 strain. 4

CDAD was diagnosed in cases with diarrhoea that were confirmed Toxin A positive. Patients with diarrhoea and demonstrable pseudomembranous colitis at endoscopy or at histopathology were also diagnosed as having CDAD. Persons under 18 years of age, and adults who provided formed stool samples to the laboratory, were excluded from the study. The Centres for Disease Control (CDC)-recommended definitions for diarrhoea were adopted. 5 Diarrhoea was considered as hospital-acquired if it had started more than 48 hours after admission, or if patients had resided in a long-term care facility, or if patients had been discharged from hospital or long-term care facility 14 days prior to presentation. CDAD was defined as diarrhoea that started before hospital admission or within 48 hours following admission.

The study was approved by the Ethics Committee of the University of Cape Town.

STATISTICAL ANALYSIS

Statistical analysis was performed using Stata Statistical Software: Release 11 (StataCorp, College Station, USA). Continuous variables were expressed as medians and interquartile ranges (all variables in the final analysis had a non-Gaussian distribution). The Mann-Whitney test was used to assess continuous variables, while the chi-square or Fisher's exact test was used for categorical variables. Univariate analysis was performed initially for each variable. Variables differing between groups with a significance level of \( p < 0.1 \) and other possible confounders identified \textit{a priori}, were then entered into a series of multivariate logistic regression models. Age was treated as a categorical variable (<30 years, 30 - 49 years, 50 - 64 years, 65+years). Models were built sequentially, starting with the variable most strongly associated with the outcome. A \( p \) value \( \leq 0.05 \) was considered significant in the multivariate model. Some baseline variables that were significant on univariate analysis were
omitted from the final models owing to collinearity. Different models were compared by using the likelihood ratio test, with significance determined at a $p$ value of 0.05.

RESULTS

A total of 651 patients were enrolled. Eight were excluded owing to incomplete data, leaving 643 patients in the final analysis. Baseline characteristics are presented in Table 1. Fifty-nine (9.2%) individuals were diagnosed with CDAD, of whom 34 (58%) were female. The diagnosis was made by toxin detection in 51 (86.4%) patients. An additional 6 (10.2%) had features of pseudomembranous colitis at endoscopy and 2 (3.4%) at histopathology. All of them had prior antibiotic exposure. None of them had an alternative cause to explain their diarrhoeal illness.

Among the CDAD cases, 39 (66%) had had recent antibiotic exposure (within 28 days of diagnosis) and 27 (46%) had been hospitalised within the previous 3 months; both variables were significantly associated with *C. difficile* infection on univariate analysis. Sixty-four per cent of patients with CDAD had been exposed to penicillin-based antibiotics — 26% to quinolones, 23% to carbapenems, 13% to cephalosporins and 3% to clindamycin. Although not statistically significant, there was a trend for increased use of proton pump inhibitors (PPIs) in subjects with CDAD compared with those without (49% v. 39%, $p=0.089$). No baseline laboratory marker was significantly associated with CDAD (Table 2). On logistic regression analysis (Table 3), only recent antibiotic exposure was identified as an independent predictor for CDAD (odds ratio (OR) 2.9; 95% CI 1.6 - 5.1).

HA-CDAD was diagnosed in 40 subjects (67.8%), 90% of whom had had recent exposure to antibiotics, giving an annual incidence of HA-CDAD of 8.7 cases per 10 000 hospitalisations. Of the 19 (32.2%) patients with CA-CDAD, only 3 (16%) reported recent exposure to antibiotics, compared with 90% with HA-CDAD ($p<0.0001$). Fourteen (24%) patients with CDAD had concomitant IBD, 12 (86%) of whom acquired the disease in the community.
(p<0.0001 v. HA-CDAD). Table 4 compares the characteristics of HA-CDAD with CA-CDAD. Subjects with HA-CDAD had a significantly lower median baseline haemoglobin (8 g/dl v. 12 g/dl, p<0.0001) and serum albumin (24 g/l v. 41.5 g/l, p<0.0001) as well as significantly higher C-reactive protein levels (83.5 mg/l v. 4 mg/l, p=0.002) than subjects with CA-CDAD. There was no difference in baseline white blood cell counts or serum creatinine. On regression analysis, only recent antibiotic exposure (OR 0.04, 95% CI 0.01 - 0.24) and the presence of comorbid IBD (OR 9.6, 95% CI 1.15 - 79.8) were predictors of CA-CDAD.

Seven (12%) patients with CDAD underwent colectomy (Table 5), which on regression analysis was significantly associated with C. difficile infection (OR 6.83; 95% CI 2.41 - 19.3). The median time between diagnosis of CDAD and colectomy was 4 days (IQR 1.75-10.75). However, no difference was observed in duration of hospital stay or the need for ICU care between subjects with and without C. difficile infection. There was no significant difference in mortality rates. Of the 59 patients with CDAD, 12 (20%) patients died. Eight (14%) patients died within 30 days of diagnosis, representing 66.7% (8/12) of the patients with CDAD. Two (3.4%) cases of HA-CDAD owing to NAP1 were identified, both of which proved fatal.

Forty-four (74.6%) patients with CDAD were treated with metronidazole, 3 (5.2%) with oral vancomycin and 8 (13.4%) with a combination of oral vancomycin and metronidazole. Four (6.8%) patients received no antibiotics directed at C. difficile, 2 of whom underwent colectomy. Of the 12 patients who died, 5 (42%) were treated with metronidazole only, 1 (8%) was treated with vancomycin only, 3 (25%) patients were treated with a combination of vancomycin and metronidazole, 3 (25%) did not receive any antimicrobial therapy directed at C. difficile. Of these 3 untreated patients, one died within the next day after admission.

Three (5.1%) patients had recurrent disease, all being successfully treated with antibiotics.
DISCUSSION

Over the last decade, there has been concern regarding the rising incidence, disease severity and mortality associated with CDAD. The number of reported cases in the United Kingdom rose from 7,470 in 1994 to 43,682 in 2004. Stoke Mandeville Hospital reported 2 outbreaks between 2003 and 2005 that resulted in 38 deaths.\(^6\) Similarly in Quebec, Canada, the overall incidence of CDAD quadrupled in 2003, with a reported 30-day mortality of 6.9% in one study.\(^7\) This markedly increased incidence has been attributed to the NAP1 strain of \textit{C. difficile} that carries a mutation in the tcd C gene, a negative regulator of toxin production, as a result of which the NAP1 strain produces more than 10 times the quantity of toxin than conventional strains.\(^3\) NAP1 also produces an additional toxin, the so-called Binary toxin, which may act synergistically with Toxins A and B in causing disease.\(^8,9\)

In southern Africa, data regarding \textit{C. difficile} is scarce. Samie \textit{et al.} undertook PCR detection of \textit{C. difficile} in adults attending an outpatient department and in school-going children in the Vhembe District, South Africa.\(^10\) They found a prevalence of 14% of \textit{C. difficile} among study participants. However, the incidence of CDAD in SA hospitals remains undetermined. In 2008, Lekalakala \textit{et al.} reported on an increase in CDAD at their tertiary hospital in Pretoria and highlighted the importance of preventive measures involving close co-ordination between the laboratory and infection control teams.\(^11\) Whether this apparent increase was caused by the NAP1 strain was not evaluated.

Overall, the annual incidence of HA-CDAD was 0.87 cases per 1,000 hospitalisations, which is far lower than Western experience, where CDAD incidence rates of 7.4 cases per 1,000 admissions have been reported.\(^12\) A possible explanation for this lower burden of disease may be the generally younger SA hospital patient population. The median age in our study was 41 years. However, when comparing patients 65 years and older with those 30 years and younger, an increased risk of developing CDAD was not observed (OR 0.7, 95% CI 0.3 -1.8).
Another possible explanation may be the strict antimicrobial stewardship programme adopted at GSH, given that limiting the use of antibiotics has been shown to decrease the occurrence of CDAD.\(^{13,14}\) The possibility remains that all cases of \textit{C. difficile} might not have been identified, and the incidence of CDAD underestimated. Stool EIA for Toxin A lacks sensitivity and may miss cases producing toxin B only.\(^{4,9}\) On the other hand, the practice of sending more than one stool specimen for EIA could have overestimated the true incidence of disease. In our study, 41 patients were diagnosed on a single stool sample, 13 patients on a second sample, 3 patients on third sample and 1 patient had 5 samples sent. Overall, the diagnosis of CDAD was made on an average of 1.4 samples. Another study limitation is that our hospital is a large, urban, tertiary-care teaching facility that may not be representative of other institutions.

Several risk factors render patients vulnerable to CDAD. The only independent variable in this study that was found to be strongly associated with CDAD, was recent exposure to antibiotics.\(^{15-17}\) The hospital milieu serves as an important reservoir for resistant spores that may be passed on to individuals who then develop disease some time after exposure. Although univariate analysis suggested an increased risk of CDAD in subjects previously admitted to a healthcare facility within 90 days \((p=0.014)\), significance was lost on multivariate analysis. SA is a high HIV-prevalent country, yet CDAD was not associated in this study with HIV infection. The relationship between CDAD and PPIs remains controversial,\(^{18}\) but their use was shown not to significantly increase the risk for CDAD in this study.

While an elevated white cell count, hypo-albuminaemia, impaired renal function and raised inflammatory markers have been associated with severe CDAD, we did not find these parameters to be of significance. However, it bears noting that our control group also had serious illnesses with abnormal inflammatory and other markers. Similarly, the length of
hospital stay, need for ICU admission, and mortality as outcome of disease did not reach significance. It is possible that this could be due to selection bias with our controls being recruited from a large tertiary institution. It is important to note that 1 in 5 patients who developed CDAD during the course of their illness eventually died. Subjects with CDAD had an almost seven-fold increase in colectomy.

The recurrence rate of CDAD was low in our study when compared to the West. An initial false positive result could explain a low recurrence rate in our study. However, the high rate of recurrence in Western countries could also be attributed to the fact that they experienced an outbreak with a more virulent strain, namely NAP1 which is more difficult to treat and has more virulent factors that favours recurrence. In comparison, we only identified two cases of NAP1. In addition, the recurrence rate has been shown to be higher in elderly patients compared to younger patients.\(^3\) The median age of patients in our study was 39 years and it is possible that younger patients are less likely to relapse. The locally prevalent strains may still be sensitive to metronidazole which was the antibiotic most commonly used to treat CDAD in our cohort, thereby explaining a low recurrence rate.

Several reports have highlighted an increased incidence of CDAD in patients with IBD.\(^{19-23}\) As in our study, it has been noted that IBD patients are particularly prone to CA-CDAD, even during remission and often without prior exposure to antibiotics.\(^{24}\) Although this is still a controversial issue, the use of maintenance immunosuppression has been found to be a significant risk factor predisposing IBD patients to CDAD.\(^{25}\) In our study, of the 14 (24%) IBD-CDAD patients, 5 (35%) were on immunosuppressant agents. In addition, colonic disease predisposes IBD patients to CDAD and patients with UC are more prone compared to Crohn’s disease.\(^{24}\) It has also been suggested that the risk increases with disease activity.\(^{25}\) However, the relationship between CDAD and disease activity remains unclear.
In conclusion: This is the first study, to our knowledge, that prospectively documents the impact of CDAD in a southern African hospital. The burden of disease is significantly lower than in the West, perhaps because the NAP1 strain has fortunately not reached the levels reported in countries recently experiencing severe outbreaks.

ACKNOWLEDGEMENTS

The authors thank Professor Sandie Thomson for reviewing the manuscript, Henri Carrara and Motasim Badri for statistical input, and the Gastroenterology Foundation, Discovery Foundation, and Medical Research Council of South Africa for funding support.

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<td>Haemoglobin (g/dl)</td>
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<td>Median 31 (IQR 24 - 39)</td>
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<td>203</td>
<td>13.3</td>
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### Table 4. Characteristics of HA-CDAD and CA-CDAD at diagnosis

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<td><strong>Race (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>4 (10%)</td>
<td>1 (5%)</td>
<td>0.18</td>
</tr>
<tr>
<td>Black</td>
<td>14 (35%)</td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td>Coloured</td>
<td>21 (53%)</td>
<td>15 (79%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (2%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Previous admission (%)</strong></td>
<td>19 (48%)</td>
<td>8 (42%)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Recent antibiotic use (%)</strong></td>
<td>36 (90%)</td>
<td>3 (16%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>PPI use (%)</strong></td>
<td>21 (53%)</td>
<td>8 (42%)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Comorbid conditions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (2%)</td>
<td>3 (16%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>14 (35%)</td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td>8 (20%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>IBD</td>
<td>2 (5%)</td>
<td>12 (63%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other</td>
<td>13 (33%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Any comorbid condition</td>
<td>39 (98%)</td>
<td>16 (84%)</td>
<td></td>
</tr>
<tr>
<td><strong>Type of IBD (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1 (50%)</td>
<td>5 (42%)</td>
<td>0.83</td>
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<tr>
<td>Crohn’s disease</td>
<td>1 (50%)</td>
<td>7 (58%)</td>
<td></td>
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</table>
Table 5. Length of hospital stay, need for ICU care and outcome of cases with CDAD and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>C. diff +ve</th>
<th>C. diff -ve</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration of hospital stay (days)</td>
<td>19 (IQR 8 - 29)</td>
<td>17 (IQR 7 - 33)</td>
<td>0.7</td>
</tr>
<tr>
<td>ICU admission</td>
<td>17 (37%)</td>
<td>116 (28%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Colectomy</td>
<td>7 (12%)</td>
<td>13 (2%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Death</td>
<td>12 (20%)</td>
<td>92 (16%)</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Colonoscopic appearance of pseudomembranous colitis - characteristic yellowish-white plaques adherent to an inflamed but intact colonic mucosa
APPENDIX 1.2 – TOXIC MEGACOLON

Toxic megacolon – dilated colon with colonic diameter exceeding 5.5cm
Lower arrow – Focal ulceration of colonic mucosa

Upper arrow – Exudation of pseudomembrane depicting a “summit” lesion

Colectomy specimen showing numerous pseudomembranes

AUTHOR GUIDELINES
Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, and will delay publication.

AUTHORSHIP
Named authors must consent to publication. Authorship should be based on substantial contribution to: (i) conception, design, analysis and interpretation of data; (ii) drafting or critical revision for important intellectual content; and (iii) approval of the version to be published. These conditions must all be met (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org).

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Authors must declare all sources of support for the research and any association with a product or subject that may constitute conflict of interest.

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Provide evidence of Research Ethics Committee approval of the research where relevant.

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References to ethnic classification must indicate the rationale for this.

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Shorter items are more likely to be accepted for publication, owing to space constraints and reader preferences.

Research articles (previously 'Original articles') not exceeding 3 000 words, with up to 6 tables or illustrations, are usually observations or research of relevance to clinical medicine and related fields. References should preferably be limited to no more than 15. Please provide a structured abstract not exceeding 250 words, with the following recommended headings: Background, Objectives, Methods, Results, and Conclusion.

Scientific letters will, in future, be incorporated as shorter Research articles.

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Refer to articles in recent issues for the presentation of headings and subheadings. If in doubt, refer to 'uniform requirements' - www.icmje.org.

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Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.

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Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

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Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'. Figure legends: Fig. 1. 'Title...'

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Authors must verify references from the original sources. Only complete, correctly formatted reference lists will be accepted. Reference lists must be generated manually and not with the use of reference manager software.

References should be inserted in the text as superscript numbers, e.g. These regulations are endorsed by the World Health Organization,2 and others.3,4,6

All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order). Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.

Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al. First and last page, volume and issue numbers should be given.

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Book references:


Chapter/section in a book:


Internet references:


Other references (e.g. reports) should follow the same format:

Author(s). Title. Publisher place: publisher name, year; pages.

Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.

Unpublished observations and personal communications in the text must not appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.

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ETHICS APPROVAL LETTER

UNIVERSITY OF CAPE TOWN

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Research Ethics Committee
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Observatory 7925
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e-mail: kanees.en@med.ac.za

12 August 2008

REC REF: 310/2008

Dr M N Rajabally
Gastrointestinal Clinic
E23, GSH

Dear Dr Rajabally

PROJECT TITLE: A PROSPECTIVE STUDY OF CLOSTRIDIUM DIFFICILE INFECTION TO INVESTIGATE THE IMPACT OF THE NAP 1 STRAIN IN A TERTIARY REFERRAL HOSPITAL

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has formally approved the above-mentioned study.

Approval is granted for one year till the 15th August 2009.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close out the file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC, REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637
Institutional Review Board (IRB) number: JRB00001938

Signed
FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)
This serves as notification of annual approval, including any documentation described below.

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<th>Annual progress report</th>
<th>Approved until/next renewal date</th>
<th>15 Jan 2014</th>
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<tr>
<td>Not approved</td>
<td>See attached comments</td>
<td>Date Signed</td>
<td>13/1/2013</td>
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Signature Chairperson of the HREC

Principal Investigator to complete the following:

1. Protocol information

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<thead>
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<td>310/2008</td>
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<td>Current Ethics Approval was granted until</td>
<td>2009</td>
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</table>

**Protocol title:**
A prospective study of Clostridium difficile infection to investigate the impact of the NAP1 strain in a tertiary referral hospital

**Protocol number (if applicable):**
N/A

**Principal Investigator:**
Naayil Rajabally

**Department / Office Internal Mail Address:**
Division of Gastroenterology – E23
Department of Medicine

1.1 Does this protocol receive US Federal funding?  □ Yes  □ No
1.2 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.  □ Yes  □ No

2. List of documentation

RESEARCH ETHICS COMMITTEE

2013-01-11
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

28 July 2012
Page 1 of 5

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)
AUTHOR CONTRIBUTIONS TO STUDY

Rajabally N – study concept and design, obtained funding, acquisition of data, analysis and interpretation of data, statistical analysis and drafting of the manuscript

Pentecost M – data capturing, administrative support, revision of the manuscript

Pretorius G – contribution to study design, material support

Whitelaw A – acquisition of data, technical support, revision of the manuscript

Mendelson M – contribution to study concept, revision of the manuscript

Watermeyer G – study concept and design, analysis and interpretation of data, statistical analysis, revision of the manuscript for important intellectual content, study supervision

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Shaheen Emambux for proofreading

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