CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHOEA AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

AMAL SALIM AWADH SALIM

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DECLARATION

Declaration by Candidate:

This thesis is submitted to the School of Medicine as part of the fulfilment of the requirements of the Master of Medicine degree in Internal Medicine, Moi University.

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Amal Salim Awadh Salim

Signature:……Date ……

Declaration by Supervisors

This thesis has been submitted for examination with our approval as Moi University supervisors.

Dr. Adrian Gardner, MD, MPH
Executive Field Director, AMPATH Consortium
Assistant Professor of Clinical Medicine, Infectious Diseases, Indiana University School of Medicine
Visiting Lecturer, Moi University School of Medicine (Kenya)
Assistant Professor of Medicine (Adjunct), Alpert Medical School of Brown University
Signature:……Date ……

Dr. Kirtika Patel, Ph.D.

Head of Immunology Department

Moi University School of Medicine

Signature:……Date ……

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DEDICATION

This thesis is dedicated to all my family members, especially my late loving mother, Jamila Mohammed, who has inspired me to be a doctor, my father, Mr. Salim Awadh, my sister, Dr. Ibtisam Salim, and my two brothers, Dr. Mohammed Salim and Mr. Adil Salim, who have always been supportive throughout this journey. Finally, to all my teachers who over the years have moulded me to become a better person and doctor.

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ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
C. difficile	*Clostridium difficile*
CCU	Cardiac Care Unit
CDAD -	*Clostridium difficile*-associated Diarrhoea Negative
CDAD +	*Clostridium difficile*-associated Diarrhoea Positive
CDAD	*Clostridium difficile*-associated Diarrhoea
CEO	Chief Executive Officer
CHG	Chlorhexidine
DM	Diabetes Mellitus
EIA	Enzyme immunoassay
GDH	Glutamate dehydrogenase
H2RA	Histamine-2 Receptor Antagonists
HIV	Human Immunodeficiency Virus
hrs	Hours
HTN	Hypertensive
ICU	Intensive Care Unit
IREC	Institutional Review and Ethics Committee
MTRH	Moi Teaching and Referral Hospital
MUSOM	Moi University School of Medicine
NAAT	Nucleic acid amplification test
PCR	Polymerase Chain Reaction
PPIs	Proton-pump Inhibitors
SPC	Sample –Processing Control
U. S	United States

OPERATIONAL DEFINITIONS

Diarrhoea:

WHO defines diarrhoea as the passage of three or more loose or liquid stools per day.

***C. difficile*–associated diarrhoea:**

Usually a hospital acquired infection defined by the presence of diarrhoea and a positive assay for *C. difficile* toxin.

***Clostridium difficile*-associated diarrhoea positive:**

Defined as participants having *Clostridium difficile*-associated diarrhoea

***Clostridium difficile*-associated diarrhoea negative:**

Defined as participants not having *Clostridium difficile*-associated diarrhoea

Hospital acquired diarrhoea/ Nosocomial diarrhoea:

An acute episode of diarrhoea in a hospitalized patient that was not present on admission and arises after ≥48 hours of hospitalization.

Clinical characteristics: Defined by the following: Age, sex, type of antibiotic used, duration of antibiotic use, admitting ward, admitting diagnosis, duration of hospitalization, HIV status, history of comorbidities, usage of gastric acid inhibitors

Admitting ward: The ward the participant was admitted to.

Admitting diagnosis: Diagnosis during admission. 1 admitting diagnosis; participant had only one diagnosis during admission. >1 admitting diagnosis; more than one diagnosis during admission

Duration of hospitalization: Number of days from the time of admission to the day of study recruitment

Gastric acid inhibitors: Proton-pump inhibitors and histamine-2 receptor antagonists

ABSTRACT

Background: *Clostridium difficile* (*C. difficile*) is a spore-forming, toxin-producing, gram-positive anaerobic bacterium. It is the leading cause of nosocomial diarrhoea. *C. difficile*-associated diarrhoea (CDAD) is one of the most common healthcare-associated infections and a significant cause of morbidity and mortality among hospitalized patients. The prevalence rate of *C. difficile* infection/carriage in sub-Saharan Africa has been reported to range from 4.9 – 43%. Despite widespread access to over-the-counter antibiotics in Kenya coupled with high rates of nosocomial diarrhoea, patients are not tested for CDAD due to presumptions of low prevalence and technical inaccessibility.

Objective: To determine the prevalence and describe the characteristics of patients with *Clostridium difficile*-associated diarrhoea (CDAD) at Moi Teaching and Referral hospital (MTRH), Eldoret, Kenya.

Methods: In this cross-sectional study, 110 unformed stool samples were taken from the participants who were admitted and developed diarrhoea after 48 hours of antibiotic use or admitted with diarrhoea with a history of antibiotics use in the last 30 days. The samples were tested for *C. difficile* toxin B and the hypervirulent strain (027/NAP1/BI strain) by using the Cepheid GeneXpert® molecular method. Participants' age, sex and clinical characteristics were analysed using Chi-square test, Fisher's exact test and Wilcoxon rank sum test. A p value <0.05 was considered to be statistically significant.

Results: One hundred and ten participants provided stool samples, of which 104 had valid PCR results. A majority (57.7%) of the participants were female with a median age of 39.5 years (IQR 25). CDAD was diagnosed in 23 (22.2% (95% CI 14.0- 30.2)) of the 104 participants whereas the hypervirulent strain was not detected in any of the samples. The median age of participants with CDAD was 42 years (IQR, 22) and 13 out of the 23 (56.5%) were female. There was a statistically significant association of CDAD with, prolong duration of hospitalization (p=0.02, Wilcoxon ranksum test); prolonged exposure to antibiotics ≥1 week (p=0.031, Fisher's exact test); exposure to >1 antibiotic (p=0.004, Fisher's exact test) and admission to surgical ward (p=0.01, Fisher's exact test). Both univariate and multivariate analysis showed that use of >1 antibiotic (OR, 3.3; 95% CI, 1.03-10.01; p= 0.04) was significantly associated with CDAD.

Conclusion: CDAD is present in our setting, with a prevalence of 22.2%. CDAD is significantly associated with long duration of hospitalization, history of prolonged antibiotic exposure and use of multiple antibiotics.

Recommendation: The diagnosis of *C. difficile* and testing for *C. difficile* toxins should be considered in persons who develop diarrhoea after history of antibiotic use.

CHAPTER ONE: INTRODUCTION

1.1 Background

Clostridium difficile is a spore-forming, toxin-producing, gram-positive anaerobic bacterium and is considered to be among the normal flora of the gastrointestinal tract. It becomes pathogenic when it colonizes the human intestinal tract after the normal gut flora has been altered. Use of antibiotics is the major risk factor for *Clostridium difficile*-associated diarrhoea (CDAD) (Owens, Donskey, Gaynes, Loo, & Muto, 2008). Other risk factors include advanced age, prolonged hospitalisation, usage of gastric acid inhibitors, history of immunosuppression and history of severe underlying disease.

C. difficile can exist in spore and vegetative forms. Outside the colon, it survives in spore form. Once spores are in the colon, they convert to their fully functional vegetative, toxin-producing form.

C. difficile is a major cause of nosocomial, antibiotic-associated diarrhoea. Recently, it has been considered as the most common bacterium causing healthcare-acquired infections in community hospitals surpassing methicillin-resistant *Staphylococcus aureus* (Miller et al., 2010).

Clostridium difficile-associated diarrhoea (CDAD) is one of the most common healthcare-associated infections and a significant cause of morbidity and mortality among hospitalized patients in resource rich countries (Allegranzi & Pittet, 2009; Kyne, Hamel, Polavaram, & Kelly, 2002; Loo et al., 2005). Its clinical features range from asymptomatic, mild diarrhoea to pseudomembranous colitis or toxic megacolon and even death (Owens et al., 2008). Unfortunately, in most of the resource limited

countries there is widespread, unregulated antibiotic use, and yet the prevalence of CDAD is unknown.

The prevalence rate of *C. difficile* infection/carriage in sub- Saharan Africa has been reported to range from 4.9% to 43% (Emeruwa & Oguike, 1990; Janssen et al., 2016; Onwueme et al., 2011) In Ghana , Tanzania, Malawi and Zambia, it was found to be 5% ,6.4%, 14% and 14% respectively (Beadsworth et al., 2014; Janssen et al., 2016; Simango & Uladi, 2014; Zulu, Kelly, Mwansa, Veitch, & Farthing, 2000). Among HIV-infected inpatients and outpatients in Nigeria, it was found to be 43% and 14% respectively (Onwueme et al., 2011). In South Africa there have been 3 different studies with the prevalence ranging from 9.2% - 17.2% (Lekalakala, Lewis, & Hoosen, 2010; Rajabally et al., 2013; Samie et al., 2008).

According to a study done to describe the characteristic and diagnosis of patients admitted from AMPATH clinic showed that 11% of the patients admitted had diarrhoea (Siika et al., 2008).

In Kenya, a study by Mwachari et al (2003), using the cell cytotoxic neutralisation assay reported a prevalence of 0% *Clostridium difficile*-associated disease (CDAD) in HIV patients who were admitted with chronic diarrhoea in Kenyatta National Hospital(Mwachari et al., 1998). Therefore, for the first time this study reports the occurrence and describes the characteristics of patients with CDAD at Moi Teaching and Referral Hospital (MTRH).

PCR is considered a superior test in detection of *C. difficile* toxins compared to the enzyme immunoassays (Chotiprasitsakul et al., 2012), it is also recommended as the most sensitive test by the IDSA latest guidelines, 2017 (L Clifford McDonald et al., 2018).

The most critical initial treatment step for CDAD is to cease administration of the antibiotic that is suspected to cause CDAD, prompt diagnosis, start on antibiotics therapy for CDAD treatment and enhanced infection control measures including hand hygiene and contact precautions by use of protective gears including gown, and gloves.

1.2 Problem statement

C. difficile is a major cause of nosocomial, antibiotic-associated diarrhoea. Worldwide, the prevalence and incidence of CDAD is noted to be increasing and also of a significant cause of morbidity and mortality among hospitalized patients in resource rich countries (Allegranzi & Pittet, 2009; Kyne et al., 2002; Loo et al., 2005; Warny et al., 2005). Additionally, CDAD increases healthcare cost. In the USA alone, it is estimated that CDAD is responsible for over US$ 4.8 billion in excess healthcare costs per year (Erik R Dubberke & Olsen, 2012).

Despite widespread access to over-the-counter antibiotics in Kenya and high rates of nosocomial diarrhoea, patients are not tested for CDAD due to presumptions of low prevalence and inaccessibility to the technology required to make the diagnosis.

There is inappropriate use of antibiotics in treatment of diarrheal diseases in Kenya and in other African countries. Despite widespread use of cephalosporins, a risk factor for CDAD, there is limited data regarding CDAD in Kenya.

Given that we have scarce data in Kenya there was a need to do a study on the prevalence of CDAD.

1.3 Justification

While the global burden of CDAD is increasing, the burden in MTRH is unknown. This study aimed to fill the existing knowledge gap by providing information on the local prevalence of CDAD at MTRH, a tertiary referral hospital, and describing the clinical characteristics of the participants having CDAD. As a result, the data obtained will be used as a baseline to access future interventions.

1.4 Research questions

This study aimed to answer the following questions:

1. What is the prevalence of CDAD among inpatients with diarrhoea at MTRH?
2. What are the clinical characteristics describing study participants with CDAD in MTRH?

1.5 Study objectives

1.5.1 Broad objective:

To determine the prevalence and describe the characteristics of participants with *Clostridium difficile*-associated diarrhoea (CDAD) at Moi Teaching and Referral hospital (MTRH), Eldoret, Kenya.

1.5.2 Secondary objectives:

1. To determine the prevalence of CDAD among participants admitted at Moi Teaching and Referral hospital (MTRH), Eldoret, Kenya
2. To describe the clinical characteristics of the participants having CDAD, in Moi Teaching and Referral hospital (MTRH), Eldoret, Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Characteristics of *Clostridium difficile*?

Clostridium difficile is an anaerobic gram-positive, spore-forming, toxin-producing bacillus which is widely found in the environment; especially in the soil, water and animal faeces. It replicates exclusively in the lumen of the gastrointestinal tract. It spreads indirectly via the faecal-oral route through spores left on surfaces.

C. difficile can exist in spore and vegetative forms. Outside the colon, it survives in spore form; spores are resistant to heat, acid, and antibiotics. The spores can survive in a hospital environment for months. Once spores are in the colon, they convert to their fully functional vegetative, toxin-producing forms and become susceptible to killing by antimicrobial agents.

C. difficile was first described in 1935 by Hall and O'Toole, and it was initially observed as a component of the intestinal flora in healthy new-borns (Hall C, 1935). At first, the bacterium was named ‘‘*Bacillus difficilis*.’’ Meaning "difficult clostridium" because of difficulty related to its isolation and growth on conventional media. Later in 1970s, the name was changed to *Clostridium difficile.*

C. difficile's pathogenic role was first appreciated in the 1970s, years after the discovery of penicillin, when *C. difficile* toxin was observed in the stools of patients with antibiotic-associated pseudomembranous colitis (Bartlett, Moon, Chang, Taylor, & Onderdonk, 1978; Noguchi & Takahashi, 1992). This organism is now recognized as the main cause of pseudomembranous colitis and other variant forms of diarrhoea and colitis in patients exposed to antibiotics. *C. difficile* is responsible for a total of 15-25% of all cases of antibiotic-associated diarrhoea and colitis.

2.2 Epidemiology of CDAD

Clostridium difficile-associated diarrhoea is the number one cause of infectious diarrhoea among healthcare workers in healthcare facilities (Bartlett, 2002; Braun, Hundsberger, Leukel, Sauerborn, & von Eichel-Streiber, 1996; S. H. Cohen et al., 2010). Recently, *C. difficile* has surpassed methicillin-resistant *Staphylococcus aureus* as the most common bacterium causing healthcare-acquired infections in community hospitals (Miller et al., 2010). Initially, *Clostridium difficile* infection was mostly associated with nosocomial infection, but currently there have been a rise in recognition of community acquired infection (Dumyati et al., 2012; Khanna et al., 2012).

Recent studies showed an increase in incidence rate of CDAD in resource rich countries. Most of these cases are reported to have an increase in morbidity and mortality (Kyne et al., 2002; Loo et al., 2005; Warny et al., 2005). In a multi-country European surveillance study, it was observed that CDAD ranged from 4-39% (Bauer et al., 2011). Moreover, in a multicenter surveillance study by the Centers for Disease Control (CDC) in the United States, found CDAD to range from 7-20% (J. Cohen et al., 2013). According to the most recent meta-analysis, the pooled *Clostridium difficile* rate was 14.8% among all participants tested in Asia and it was 16.4% among hospitalized participants with diarrhoea and the incidence and mortality rates are comparable to the western countries (Borren, Ghadermarzi, Hutfless, & Ananthakrishnan, 2017).

The increase in morbidity and mortality was associated with the presence of the virulent strain, 027/NAP1/BI strain, which is responsible for the previous epidemic outbreak in western countries (Loo et al., 2005; L. C. McDonald et al., 2005). The

027/NAP1/BI strain is more virulent than other strains and is also known as the hypervirulent strain (Miller et al., 2010). In Canada, the incidence increased from 3 to 12 per 1000 persons (1991 to 2002) to 25 to 43 per 1000 persons (2003 to 2004) among admitted patients (Pepin, Valiquette, & Cossette, 2005). CDAD has been associated with increase in healthcare costs which suggest that CDAD is a significant burden on the healthcare system (Erik R Dubberke & Olsen, 2012; Kyne et al., 2002). In the USA alone, it is estimated that CDAD is responsible for over US$ 4.8 billion in excess healthcare costs per year (Erik R Dubberke & Olsen, 2012). Moreover, CDAD is now globally known as a common fatal hospital and community acquired infection (Dumyati et al., 2012).

Despite Asia having comparable rates of CDAD with U.S. and Europe, the prevalence of hypervirulent strain is lower (0.3%) (Borren et al., 2017) in comparison to 21% in England (M. Wilcox et al., 2012) and 19% in European study (Davies et al., 2014).

The prevalence rate of *Clostridium difficile* infection/carriage in sub- Saharan Africa has been reported to range from 4.9% to 43% (Emeruwa & Oguike, 1990; Janssen et al., 2016; Onwueme et al., 2011). Among HIV infected participants, both Kenya and Zambia found a prevalence of 0% (Mwachari et al., 1998; Zulu et al., 2000), while Malawi and Nigeria found 14% and 43% respectively (Beadsworth et al., 2014; Onwueme et al., 2011). In other studies, where participants who had diarrhoea were investigated, they found the prevalence in Ghana, Tanzania, Zimbabwe and South Africa to be 5%, 6.4% , 8.6% and 14% respectively (Janssen et al., 2016; Samie et al., 2008; Seugendo et al., 2015; Simango & Uladi, 2014). Moreover, the three other studies done in South Africa by Rajabally et al (2013), Kullin et al (2015) and Lekalakala et al (2010), found a prevalence of 9.2%, 16% and 17.2% respectively (Kullin et al., 2015; Lekalakala et al., 2010; Rajabally et al., 2013). In Uganda, the

prevalence of CDAD was 2.4% among children admitted with diarrhoea in Mulago Hospital (Khainza, 2014). In addition, *C. difficile* was isolated in 29.0% of chicken faeces samples and in 22% of soil samples in Zimbabwe (Simango & Mwakurudza, 2008).These reservoirs of *C. difficile*, may act as a source of human infection.

There are only three studies in sub Saharan Africa that checked on the profile of toxigenicity of the strains present in the stools of participants having CDAD. There was only one study in South Africa by Samie et al (2008), that found a prevalence of 4.4% of hypervirulent strain among CDAD participants (Samie et al., 2008).

2.3 Pathogenesis and life cycle of *Clostridium difficile*

C. difficile, has an interesting life cycle that ensures its success. Although anaerobic, the spore form of *C. difficile*, can survive for months in aerobic settings. The spore structure contains several layers, including an exosporium, coat, cortex, membrane, and a DNA-containing core (Lawley et al., 2009). As a result of its structure, the spore is resistant to alcohol-based cleaning agents used commonly in hospitals.

Pathogenesis involves germination of spores into vegetative cells, colonization within the gut microbiota, and production of toxins which lead to toxin-induced intestinal damage and inflammation. Once ingested, the multiple layers of the spore help protect it from stomach acid and digestive enzymes. Spore germination occurs upon interaction with the appropriate germinants within the intestinal tract, which include taurocholic acid, a taurine-conjugated bile acid, and glycine (Giel, Sorg, Sonenshein, & Zhu, 2010; Howerton, Ramirez, & Abel-Santos, 2011). CspC, the receptor for taurocholic acid on a *C. difficile* spore, was recently discovered (Francis, Allen, Shrestha, & Sorg, 2013), and researchers demonstrated that changed germination dynamics was due to mutations in this protein. The germination normally occurs

within the cecum and colon avoiding the small intestine, which has factors that suppress wide-scale germination (Howerton et al., 2011).

Subsequently, *C. difficile* initiates the activation of the pathogenicity locus in the genome. The pathogenicity locus, approximately 19.6 kb in size, is composed of 5 genes, *tcdA, tcdB, tcdC, tcdR,* and *tcdE,* which are responsible for the production of two large clostridial toxins, A (TcdA) and B (TcdB) (Metcalf & Weese, 2011; Voth & Ballard, 2005). The gene *tcdR,* is a positive regulator of gene expression whereas *tcdC* is a negative regulator. A pore-forming holin is encoded by *tcdE,* which encodes a protein which is essential for the release of toxins A and B from the cell. CodY is another gene regulator, which binds and represses *tcdR* and thus inhibits toxin production, when essential nutrients are not available (Dineen, Villapakkam, Nordman, & Sonenshein, 2007). Notably, CDAD-induced colitis only occurs when either of TcdA or TcdB is present (Kelly & LaMont, 2008). Patients with pseudomembranous colitis have toxin for *C. difficile* in > 96% of the cases (Bartlett, 1994). TcdA was originally believed to be the only toxin necessary for virulence until the discovery of TcdA-/TcdB+ *C. difficile* strain in major breakouts worldwide (Freeman et al., 2010). On the other hand, TcdA+/TcdB- strains are equally likely to cause disease (Kuehne et al., 2010).

C. difficile causes diarrhoea through the secretion of exotoxins within the gastrointestinal tract. Toxins A and B are the major virulence factors (Taylor, Thorne, & Bartlett, 1981). Inside the cell membrane, these toxins inactivate the transformation pathway mediated by Rho family proteins, which are responsible for the proper construction of actin cytoskeleton and the signal transduction by guanosine triphosphate (GTP). This affects the cell and leads to cessation from its regular cycle

and apoptosis (Riegler et al., 1995). Both toxins affect the strength of the intercellular bonds (Brito et al., 2002).

Significant increase in toxins in the faecal load is associated with a higher severity of the illness (Akerlund, Svenungsson, Lagergren, & Burman, 2006). Toxin A causes neutrophilic infiltration and damage to the colonic mucosa, leading to an increased secretion of fluid within the digestive tract, mucosal inflammation and structural damage (Pothoulakis et al., 1988; Triadafilopoulos, Pothoulakis, O'Brien, & LaMont, 1987). Toxin B has similar destructive effects on the colonic mucosa, but appears to be roughly 10 times more cytotoxic than toxin A (Brito et al., 2002; Riegler et al., 1995).

Necrosis and sloughing of cellular debris into the colonic lumen results from the interaction of the toxins with colonocyte surface receptors that induce the degradation of actin filaments (Voth & Ballard, 2005). Toxin induced cytokine release triggers the exudation of inflammatory cells and proteins from the resulting mucosal ulcerations (Meyer et al., 2007). The resulting inflammatory exudate forms the pseudomembrane that is nearly pathognomonic for CDAD (Riegler et al., 1995).

C. difficile also produces a third toxin named the binary toxin; it has an enterotoxic activity in vitro, but its role in the pathogenicity remains unclear (Geric et al., 2006).

Additionally, there is also a hypervirulent strain, NAP1/BI/027. The strain is also known as the epidemic strain; found to be responsible for multiple hospital outbreaks of severe CDAD associated with increased morbidity and mortality in Canada, U.S. and Europe (Loo et al., 2005; L. C. McDonald et al., 2005; Warny et al., 2005). This strain was first observed at the beginning of the twenty-first century. It has been reported that this strain is more virulent compared to other pathogenic isolates (Miller et al., 2010). The complexity of the name is due to the different methods applied in

detecting the presence of the bacteria: pulsed-field gel electrophoresis (NAP1), restriction endonuclease analysis (BI) and polymerase chain reaction (027).

The hypervirulent strain has unique features that differentiate it from other strains described in the past (Loo et al., 2005; L. C. McDonald et al., 2005). Firstly, it produces binary toxins, and produces 16 to 23 times more toxins A and B in vitro (Warny et al., 2005), probably in relation to a novel 1-base pair deletion in the tcdC gene, leading to a stop codon and truncation of the tcdC protein, normally a down regulator of toxin production (MacCannell et al., 2006; Stabler, Dawson, Phua, & Wren, 2008). However, this has come into question due to recent evidence that demonstrates no change in toxin production (Cartman, Kelly, Heeg, Heap, & Minton, 2012).

Another characteristic of hypervirulent strain is toxinotype III (Toxinotyping is based on analysis of the pathogenicity locus of the *C. difficile* genome) (L. C. McDonald et al., 2005; Warny et al., 2005). Lastly, BI/NAP1/027 has acquired high-level resistance to all fluoroquinolones. Knowing that historical isolates of BI/NAP1/027 before 2001 used to be susceptible to fluoroquinolones (L. C. McDonald et al., 2005; Pepin, Saheb, et al., 2005), it is presumed that the acquisition of resistance to fluoroquinolones is as a result of transmission within hospital environments, promoting its emergence as an epidemic strain.

2.4 Patient risk factors

CDAD is the leading cause of nosocomial infectious diarrhoea (Barbut et al., 1996). In the last two decades, the dramatic increase in incidence and severity of CDAD in many countries worldwide (Clements, Magalhaes, Tatem, Paterson, & Riley, 2010) has made CDAD a global public health challenge (Eckmann, Wasserman, Latif, Roberts, & Beriot-Mathiot, 2013; Lessa, Gould, & McDonald, 2012). CDAD is now

globally known as a common fatal hospital and community acquired infection (Dumyati et al., 2012).

The most significant factor that leads to CDAD is the disruption of intrinsic colonization resistance (Britton & Young, 2012) and ingestion of the *C. difficile* spores. Factors that disrupt the intestinal microbiota allowing *C. difficile* to grow include treatment with antibiotics, proton-pump inhibitors (PPIs), and chemotherapy agents in addition to physical effects of abdominal surgery and nasogastric tubes (D. N. Gerding, Olson, Johnson, Peterson, & Lee, 1990; Loo et al., 2011). Other host factors associated with an increased risk of CDAD include advanced age, multiple comorbidities, suppressed immune system, inflammatory bowel disease and dense intestinal co-colonization with enterococci (Aronsson, Mollby, & Nord, 1985; Cefai, Elliott, & Woodhouse, 1988; Clayton et al., 2009; Ozaki et al., 2004). Besides advanced age, duration of hospitalization is one of the important risk factors for CDAD (Dale N Gerding, 1997; Pepin, Saheb, et al., 2005; Seugendo et al., 2015). The increase in chances of getting CDAD during hospitalization may be a surrogate for the duration and degree of exposure to the organism. With increase duration of hospitalization, it is more likely that the participant has a severe underlying illness and as a result a higher chance of being exposed to antibiotics. It is also worth pointing out that the observed association between advanced age and multiple comorbidities with CDAD may be confounded by medication exposure given that “polypharmacy” is common among this group of participants.

The most important and modifiable risk factor for CDAD is prior antibiotic use (Clayton et al., 2009; Hensgens, Goorhuis, Dekkers, & Kuijper, 2012). Antibiotics disrupt the competitive balance in the gut microbiota and promote the overgrowth of *C. difficile* (Chang et al., 2008). Nearly every antibiotic has been implicated in leading

to CDAD, (Kelly, Pothoulakis, & LaMont, 1994), however use of broad spectrum antibiotics with anti-anaerobic activity appear to be associated with the greatest risk (Bignardi, 1998). Before the epidemics from the hypervirulent strain, clindamycin, ampicillin, and cephalosporins were the most frequently implicated (Bignardi, 1998). With the increasing use of fluoroquinolones in hospitalized patients, these antibiotics have also emerged as an important cause of CDAD associated with the hypervirulent strain (Pepin, Saheb, et al., 2005; Stevens, Dumyati, Fine, Fisher, & van Wijngaarden, 2011).

Not only is longer exposure to antibiotics a risk factor, but also an exposure to multiple antibiotics as opposed to a single class of antibiotics, increases the risk for CDAD (Loo et al., 2005; Pepin, Saheb, et al., 2005). However, there is evidence that single-dose surgical antimicrobial prophylaxis and short courses of antibiotics may also be associated with increase of CDAD (Privitera et al., 1991).

Several literature reviews have shown that older age is a risk factor for CDAD and the risk has also been quantified (Dale N Gerding, 1997; Loo et al., 2011; Miller et al., 2010; Pepin, Saheb, et al., 2005). The severity and frequency increase with age (Miller et al., 2010). For every additional year of age after age 18, the risk of healthcare associated CDAD increases by approximately 2%. CDAD disproportionately affects older patients with dramatic differences observed in those ≥65 years of age, as evidenced by the several fold higher age-adjusted rate of CDAD in this subpopulation (S. H. Cohen et al., 2010; Pepin, Valiquette, et al., 2005). In the U.S. a total of 93% of deaths from CDAD occurred in persons ≥65 years of age and *C. difficile* was reported as the 18th leading cause of death in this age group in 2008 (Minino, 2010). The reason why advanced age is a risk factor is not clear, but it is likely multifactorial. One of the possible reasons is that older age is associated with

reduced immune response to infections. Moreover, older patients generally have other comorbidities putting them at risk of requiring multiple antibiotics or prolonged hospitalisation.

It has been noted that usage of gastric acid inhibitors such as proton-pump inhibitors (PPIs) and histamine-2 receptor antagonists (H2RA) has been associated with increase of CDAD. PPIs almost double the incidence of CDAD (Cunningham, Dale, Undy, & Gaunt, 2003; E. R. Dubberke et al., 2007) and use of H2RA is associated with less risk for CDAD than use of PPIs. The concomitant use of PPIs and antibiotics confers a greater risk than either treatment alone (Kwok et al., 2012).

It is still controversial whether use of gastric acid inhibitors is a risk factor for CDAD or not. Despite the fact that there are several studies showing that use of gastric acid inhibitors is an independent risk factor for CDAD (Cunningham et al., 2003; Dial, Alrasadi, Manoukian, Huang, & Menzies, 2004; Janarthanan, Ditah, Adler, & Ehrinpreis, 2012; Stevens et al., 2011), outcomes from other well controlled studies show that it is not the use of gastric acid inhibitors that is the risk factor for CDAD, but the association is the result of confounding by advanced age, prolong hospital stay and the history of comorbidities (Loo et al., 2005; Novack et al., 2014; Pepin, Saheb, et al., 2005; Weiss et al., 2015).

Proton-pump inhibitors may contribute to the disruption of the bowel flora, resulting in bacterial colonization of the stomach and upper small intestine (Williams, 2001). Moreover, PPIs appear to have direct effects on intestinal neutrophil activity, which may interfere with defence mechanisms protective against CDAD (Agastya, West, & Callahan, 2000; Yoshida et al., 2000; Zedtwitz-Liebenstein et al., 2002). Decreased gastric acidity can lead to inadequate sterilization of ingested organisms, as a result creating a niche for *C. difficile* colonization (Thorens et al., 1996).

In the U.S., recent healthcare exposure is a significant patient risk factor for CDAD (Freeman et al., 2010; Johnson, 2009; Pacheco & Johnson, 2013). Frequent hospitalization and increased length stay have been identified as risk factors for CDAD (Freeman et al., 2010). Contamination of *C. difficile* spores from healthcare workers' hands after caring for patients with *C. difficile*, range between 14 and 59% (L. V. McFarland, Mulligan, Kwok, & Stamm, 1989; Samore, Venkataraman, DeGirolami, Arbeit, & Karchmer, 1996). Studies have shown that within the first week, 13–20% of inpatients in a hospital acquire *C. difficile* and by 4 weeks, 50% of all in-house patients are colonized (L. V. McFarland et al., 1989; Viscidi, Willey, & Bartlett, 1981). Patients are exposed through contact with the healthcare environment or healthcare workers hands, stressing the importance of infection control and prevention efforts in the acute care setting and in other associated healthcare environments as well. The duration of hospitalisation may be a proxy for the duration and the degree of exposure to the *C. difficile*.

Compared to the immune-competent, immune-compromised patients are known to have a higher risk of CDAD. It is well known that the rate of CDAD in the post-transplant setting is higher due to the use of immune-suppressive drugs (Yanai et al., 2011). It has also been reported that cancer patients have a higher risk of CDAD compared with non-cancer patients due to chemotherapy causing the immunosuppression (Albright et al., 2007). Patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) are at a high risk of being infected with *C. difficile* too. This relationship is stronger in those who meet clinical criteria for an AIDS diagnosis or with low absolute CD4 T cell counts (Haines et al., 2013).

Populations with underlying chronic comorbid conditions have an increased risk of developing CDAD, including those with prior kidney failure (Dial et al., 2004), HIV (S. H. Cohen et al., 2010; Raines & Lopez, 2011), solid organ (Boutros et al., 2012; E. R. Dubberke & Riddle, 2009) or hematopoietic stem cell transplantation (Chopra, Alangaden, & Chandrasekar, 2010) and inflammatory bowel disease (Navaneethan, Venkatesh, & Shen, 2010).

2.5 Transmission of Clostridium difficile

The two main means of transmission of *C. difficile* are faecal-oral and environmental contamination. Patients with *C. difficile* carriage are a reservoir for environmental contamination in the presence or absence of clinical infection. *C. difficile* is highly transmissible via the faecal-oral route by ingestion of spores. Transmission occurs primarily in health care facilities, where exposure to antimicrobial drugs and environmental contamination by *C. difficile* spores are more common (Curry et al., 2013; Samore et al., 1996; M. H. Wilcox et al., 2003). *C. difficile* on the contaminated hands of healthcare workers has been extensively described (Curry et al., 2013). The organism can be cultured readily from the hospital environment, including items in patient rooms as well as the hands, clothing, and stethoscopes of healthcare workers (D. N. Gerding, Johnson, Peterson, Mulligan, & Silva, 1995; Kim et al., 1981; Samore et al., 1996; M. H. Wilcox et al., 2003). Infection is also transmitted readily between hospital roommates (L. V. McFarland et al., 1989; Samore et al., 1996).

Transmission between healthy individuals who are asymptomatically colonized has also been reported (Kato et al., 2001). These individuals are capable of shedding spores of *C. difficile* and serve as a reservoir for environmental contamination to other hospitalized patients (Curry et al., 2013; Riggs et al., 2007).

The spore-forming ability of *C. difficile* makes it distinct from other infectious organisms common to healthcare settings and introduces further challenges to reducing transmission. Spores can persist in the environment for long periods and require chlorine- or peroxide-based sporicidal agents or ultraviolet radiation devices for environmental decontamination (Boyce et al., 2008; Nerandzic, Cadnum, Pultz, & Donskey, 2010; M. H. Wilcox et al., 2003). Jabbar et al (2010) reported that after the use of 3-ml alcohol-based gel, a mean of 30% of the inoculum of *C. difficile* spores remained on hands and by handshaking the spores can be transferred to a second volunteer (Jabbar et al., 2010).

Although numerous strains coexist within a single hospital, outbreaks are typically linked to the hypervirulent strain. The spores of *C. difficile* are difficult to eradicate due to a resistance to many environmental cleaning detergents (Bury, 1992) and can be isolated from environmental swabs taken from a patient's room months after discharge (Stabler et al., 2008). Although persistence in the environment is well documented, it is typically thought to spread through person-to-person transmission. Health care workers are often responsible for spreading the organism on their hands or medical equipment (Garey et al., 2008).

2.6 Clinical manifestation of *Clostridium difficile*

Clostridium difficile-associated diarrhoea can range from asymptomatic colonization, mild-to-profuse diarrhoea with abdominal pain, to more severe disease, including pseudomembranous colitis, toxic megacolon, sepsis, and death (Bartlett & Gerding, 2008). The basis for this range of clinical manifestations is not fully understood but may be related to various host and pathogen factors.

Most cases with asymptomatic colonization can stay as carriers and do not get infected throughout their lifetime. In CDAD with colitis, watery diarrhoea is the cardinal symptom. As the illness progress, the symptoms include fevers and leucocytosis (white blood cell count ≥15,000/μL). Other manifestations include lower abdominal pain and cramping, nausea and anorexia (Bagdasarian, Rao, & Malani, 2015; Wanahita, Goldsmith, & Musher, 2002). Diarrhoea may be associated with occult blood or mucus, but melena or haematochezia are rare (S. H. Cohen et al., 2010).

Pseudomembranous colitis is the most well-known form of *C. difficile* infection. Some patients may progress to fulminant colitis, and those with severe and protracted infections can sometimes develop rectal toxic megacolon, perforation and necrosis of the intestine or rapidly progressive infections with multiple organ failure.

Severe CDAD is associated with a temperature >38.5°C, leucocytosis (white blood cell count of ≥40,000/μL), elevated creatinine, and elevated lactate.

2.7 Diagnosis of Clostridium difficile

Clostridium difficile-associated diarrhoea is diagnosed by presence of *C. difficile* toxins in loosely unformed stool. CDAD should be suspected in patients who develop acute diarrhoea with no alternative reason especially among those having possible associated risk factors for CDAD.

Several laboratory stool tests are available alone or in combination as part of a diagnostic algorithm (L Clifford McDonald et al., 2018):

1. Nucleic acid amplification test (NAAT): Its sensitivity is higher than enzyme immunoassay and cytotoxic assays. It is specific for toxigenic strains only. It can be either used alone or part of a diagnostic algorithm.

2. Enzyme immunoassay (EIA) for *C. difficile* glutamate dehydrogenase (GDH): GDH is an important enzyme produced by both toxigenic and non-toxigenic strains. It is used as an initial step in the multistep approach in a diagnostic algorithm.

3. Enzyme immunoassay for *C. difficile* toxins A and B. Testing for both toxins increases the sensitivity of EIA than testing for toxin A or B alone. Its specificity is around 99% with a sensitivity of 75%

4. Cell culture cytotoxicity assay: detects toxin directly in stool. It is more sensitive than EIA but it is limited by taking long time for diagnosis (approximately 48hrs), the requirement for a cell culture facility and lack of standardization.

5. Selective anaerobic culture: takes days to get results. It's a highly sensitive diagnostic method when a toxin test is done on the *C. difficile* isolated from the anaerobic cultures.

According to the IDSA latest guidelines (L Clifford McDonald et al., 2018):

NAAT alone or a multistep algorithm for testing (i.e. GDH plus toxin; toxins was determined by NAAT or EIA) is the most sensitive method to diagnose CDAD.

2.8 Treatment of Clostridium difficile

No treatment is recommended for asymptomatic carriers. Implementation of infection control policies like hand washing, wearing of gloves should be part of management.

According to the Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2017 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) (L Clifford McDonald et al., 2018), the treatment for CDAD is as follows:

1. Stop the therapy of the inducing antimicrobial agent(s) as soon as possible
2. Start empirical treatment as soon as the diagnosis of severe or complicated CDAD is suspected
3. The decision to start, stop, or continue treatment must be individualized if the stool toxin assay result is negative
4. Avoid use of antiperistaltic agents, if possible, as they may mask symptoms and precipitate toxic megacolon.
5. Either 10-day course of vancomycin (125mg orally 4 times per day) or fidaxomicin (200 mg twice daily) is recommended over metronidazole for first-line therapy of CDAD. The recommendation was secondary to several clinical trials that showed greater cure rate and less frequency in recurrence following vancomycin compared with metronidazole (L Clifford McDonald et al., 2018).
6. In settings where there is limited access to vancomycin or fidaxomicin, metronidazole can be used as a first-line therapy for non-severe CDI. The suggested dose is 500mg orally 3 times per day for 10 days.
7. Vancomycin administered orally, 500mg 4 times per day, is the best treatment for fulminant CDI. If the patient is having ileus, 500mg of vancomycin in approximately 100mL normal saline per rectum every 6 hours can be given as a retention enema). Intravenous metronidazole, 500mg every 8 hours, should be given with oral or rectal vancomycin especially if ileus is present.

8. Subtotal colectomy with preservation of the rectum can be done for patients who are severely ill and require surgical management. Another technique that may result to better outcome is diverting loop ileostomy with colonic lavage followed by antegrade vancomycin flushes.
9. The first recurrence of CDI is treated with:
    a. Oral vancomycin as a tapered and pulsed regimen rather than a second standard 10-day course of vancomycin, or
    b. A 10-day course of fidaxomicin rather than a standard 10-day course of vancomycin, or
    c. A standard 10-day course of vancomycin rather than a second course of metronidazole if metronidazole was used for the primary episode
10. For patients with >1 recurrence of CDI, antibiotic treatment options are:
    1. oral vancomycin therapy using a tapered and pulsed regimen,
    2. a standard course of oral vancomycin followed by rifaximin or fidaxomicin.
11. Faecal microbiota transplantation is recommended for patients with multiple recurrences of CDI who have failed appropriate antibiotic treatments
12. For patients who require continued antimicrobial therapy for the underlying infection, no recommendations can be made regarding prevention of recurrent CDAD.

2.9 Prevention of Clostridium difficile

The most important point in prevention of CDAD is protecting patients from initial acquisition of the organism in the healthcare setting. As with many other nosocomial pathogens, strict hand hygiene and appropriate contact precautions are the cornerstones of reducing the spread of *C. difficile* between patients. Although hand

rubbing with alcohol-based products is now considered as the reference method worldwide for hand hygiene, *C. difficile* spores are not eradicated by the commonly used alcohol-based hand sanitizers. A study by Jabbar et al (2010) found that a mean of 30% of the inoculum of *C. difficile* spores remained on hands after the use of 3-ml alcohol-based gel and that spores can be transferred to a second volunteer just by handshaking (Jabbar et al., 2010). The use of these products has not been associated with any significant increase in the incidence of CDAD within centers (Boyce, Ligi, Kohan, Dumigan, & Havill, 2006).

Contact precautions that include the use of a gown and gloves when entering a patient's room also result in a significant decrease in new CDAD cases (Johnson et al., 1990; Zafar, Gaydos, Furlong, Nguyen, & Mennonna, 1998). The combination of rigorous hand hygiene with contact precautions can decrease the incidence of CDAD by as much as 80% (Johnson et al., 1990; Muto et al., 2007; Zafar et al., 1998).

Chlorhexidine (CHG) bathing may reduce hospital-acquired CDAD (Rupp et al., 2012). Another strategy is to reduce inappropriate antimicrobial use through stewardship.

CHAPTER THREE: METHODOLOGY

3.1 Study design

This study is a descriptive cross-sectional study.

3.2 Study site

This study was carried out at Moi Teaching & Referral Hospital (MTRH) Eldoret, Kenya. MTRH is the second largest teaching and referral hospital in the country and serves as the teaching hospital for Moi University School of Medicine (MUSOM).

MTRH serves mainly the North Rift region plus part of western Kenya. Some patients also come from places far away such as Uganda and northern parts of Kenya. The hospital has a bed capacity of 2000.

The study was carried out at the MTRH, in both the public and private wing. The participants were recruited from the medical ward, surgical ward, gynaecological ward, Cardiac Care Unit (CCU) and Intensive Care Unit (ICU).

3.3 Study population

Patients admitted at Moi Teaching & Referral Hospital (MTRH), who were ≥18 years, on antibiotics and developed diarrhoea after 48 hours (hrs) of antibiotics use and/or patients admitted with diarrhoea with a history of antibiotics use in the last 30 days.

3.4 Sampling and sample size determination

All the participants meeting the eligibility criteria were selected using consecutive sampling technique- that is any participant qualifying for the study was recruited until the desired sample size was achieved.

The participants were identified by approaching the ward registrars or care providers. Once the participants were identified, the investigator confirmed that the identified participant meets the eligibility criteria.

In Kenya, there is one study that checked on the prevalence of CDAD among HIV participants who had chronic diarrhoea and it was 0%. However, a similar study has been carried out in Tanzania that found the prevalence of CDAD among admitted patients to be at 6.4%

Hence so as to be 95% accurate that the proportion of patients seen with CDAD is within ± 5% of the population prevalence of 6.4%. The sample size was calculated using Fisher's formula.

Fisher's formula is an appropriate formula to calculate the sample size required to get the prevalence of a disease.

Fischer et al. (1998) formula is as below:

$$n = \frac{(Z\alpha/2)^2 * p * (1-p)}{d^2}$$

Where;

n = minimum sample size required

α = the level of significance (5%)

Zα/2 = the value of Z at the selected level of significance = 1.96

p = likely prevalence (6.4%)- Tanzanian study (Mwanza)

d = P value (0.05)

$n = ((1.96^2) * 0.064 * 0.936 / (0.05)^2) = 92$

After an inflation of 16% the sample size was (92/0.16) 110 participants.

Inflation was done to overcome the invalid results. Since this is test was done for the first time in Kenya and there was a chance of getting invalid results if there is presence of inhibitors, for example blood or mucus, in the PCR reaction.

3.6 Eligibility criteria

a. Age ≥18 years.
b. Any participant admitted at the Moi Teaching and Referral Hospital, (MTRH) on antibiotics and developed diarrhoea after 48hrs of antibiotic use.
c. Any participant admitted with diarrhoea with a history of antibiotics use in the last 30 days before admission.
d. Consent given by the patient.

3.7 Study procedure

Once the participant identified met the eligibility criteria, the investigator had their consent obtained and filled in a data collection form which had details about the participant's age, admitting diagnosis, admitting ward, duration of hospitalisation, type and duration of antibiotic use, history of comorbidities, history of HIV, history use of gastric acid inhibitors and source of drinking water. Once filled, a previously unevaluated stool sample was obtained from the participants in a sterile, dry, clean and labelled container. The container was placed in a sealed biohazard labelled plastic bag to avoid contamination and was transported to the AMPATH Reference laboratory for processing and testing. All stool samples were tested for *C. difficile* toxins using real-time polymerase chain reaction (PCR) (Cepheid Xpert C. difficile/Epi, Cepheid, Sunnyvale CA) to identify toxin-producing *C. difficile* strains, toxin B and hypervirulent strain. The hypervirulent strain was confirmed by the

presence of its characteristics: Binary Toxin (*cdt*), and *tcdC* deletion 117 (*tcd*C□117). The procedure is explained in details in (Appendix III)

If the test was not performed on the same day, the sample was stored at 2–8°C until testing.

The sample was considered stable for up to 5 days when stored at 2–8°C. Alternatively, it could be kept at room temperature (20-30°C) for up to 24 hours.

Once the results were obtained, it was shared with the primary clinicians taking care of the participants. The decision about treatment was made by the primary clinicians.

The figure 1 below summarises this study procedure.

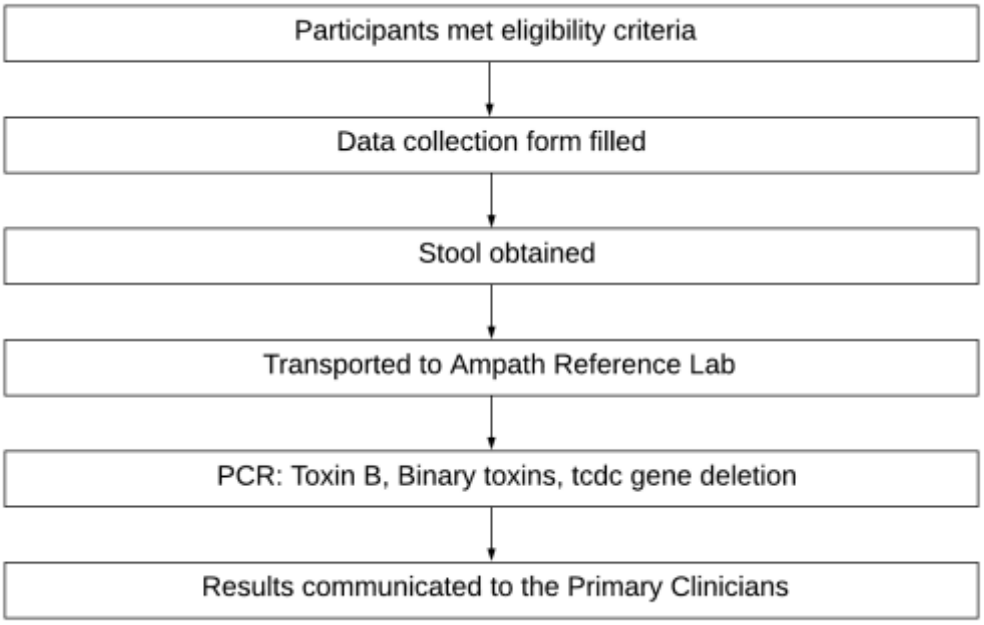

Figure 1: Study procedure

3.8. Study timeline: 4 months

3.9. Data management and analysis

3.9.1 Data collection

Data was collected between June and September 2017. The principal investigator reviewed each participant before enrolment to the study to ensure they all met the eligibility criteria.

Once a written consent for the test was obtained from the participant, data collection form was filled (Appendix III). Medical records were also reviewed, and relevant clinical data were extracted and entered into the data collection form. The variables collected included biodata such as age, gender and source of drinking water. History of comorbidities was also obtained. Other variables collected included; admitting diagnosis, admitting ward, duration of hospitalization, HIV status, class and duration of antibiotic used, history of using gastric acid inhibitor, if yes; the type and duration of the gastric acid inhibitor used. The dependent/outcome variables were the presence of *C. difficile* toxin B and hypervirulent strain for the participants who were on antibiotics and developed diarrhoea after 48hrs of antibiotics use or participants admitted with diarrhoea with a history of antibiotics use in the last 30 days.

3.9.2 Data cleaning

Data cleaning included scrutinizing completed forms for inconsistencies, errors and omissions was done on a weekly basis to ensure its completeness and to avoid inconsistencies.

3.9.3 Data entry

Data was entered in Microsoft Excel database, and quality was controlled by double checking the data entered to ensure accuracy of the data.

3.9.4 Data protection and safety

To ensure confidentiality, all participants' records were de-identified. The stored data in the computer is only available to the principle investigator via password protection. Database was also backed up for recovery when necessary.

Participants and their primary physicians taking care of them were provided with a copy of the results and the participants had the autonomy over who else can view their test results.

All raw data was kept under lock and key and will be saved for approximately 5 years for future reference if need be.

3.9.5 Data analysis

The data was analysed using STATA (Version 15). All variables were inspected for missing data, inconsistence and distribution. New variables generated included new categories for pre-existing comorbidities, antibiotic use and duration of hospitalisation.

Descriptive analysis was carried out and the results were presented in frequencies, percentages, medians and the corresponding interquartile range (IQR).

Wilcoxon rank-sum test was used to compare medians was and Pearson's Chi Square test was used to compare categorical variables. Fisher's exact test was used to compare categorical variables where numbers were small. The prevalence was calculated with 95% confidence interval and p value <0.05 was considered significant.

A sub-analysis done using a multivariate logistic regression was used to determine predictors of participants having CDAD.

3.10 Ethical considerations

This study was carried out with the approval of the Institutional Research and Ethics Committee (IREC) of MTRH and Moi University School of Medicine and permission from MRTH management (Appendix V). A signed written informed consent was obtained for each participant who was included in this study (Appendix I &II). Confidentiality was maintained throughout the study by password protecting the database and limiting access only to the principal investigator. Interviews were carried out in a consultation room to ensure privacy and convenience. All participants including those who declined consent received the same level of care afforded to all other patients. There were very minimal anticipated risks to the participants attributable to this study. Data collection for will be shredded after five years or publication of the study findings. There was no conflict of interest in this study and no incentives were used to recruit participants.

3.11 Dissemination of results

The results of the study were disseminated through a written report to the participants and their primary clinicians taking care of them. The results of this study shall be published in a reputable journal and presented in professional conferences and seminars. This thesis shall be availed at the MUSOM library.

CHAPTER FOUR: RESULTS

The recruitment period was between June 2017 – September 2017.

A total of 134 participants were approached and screened for eligibility into the study. Of these, 110 met the inclusion criteria and were successfully consented and recruited into the study. Samples were obtained from the 110 participants and were processed and tested; 6 of the 110 had invalid results. Of the 24 excluded participants: 7 declined to participate; 5 consented but were discharged before the sample was obtained for testing; 10 participants' diarrhoea had stopped; 2 of the participants died before the sample was obtained.

The enrolment schema is as shown in figure 2 below.

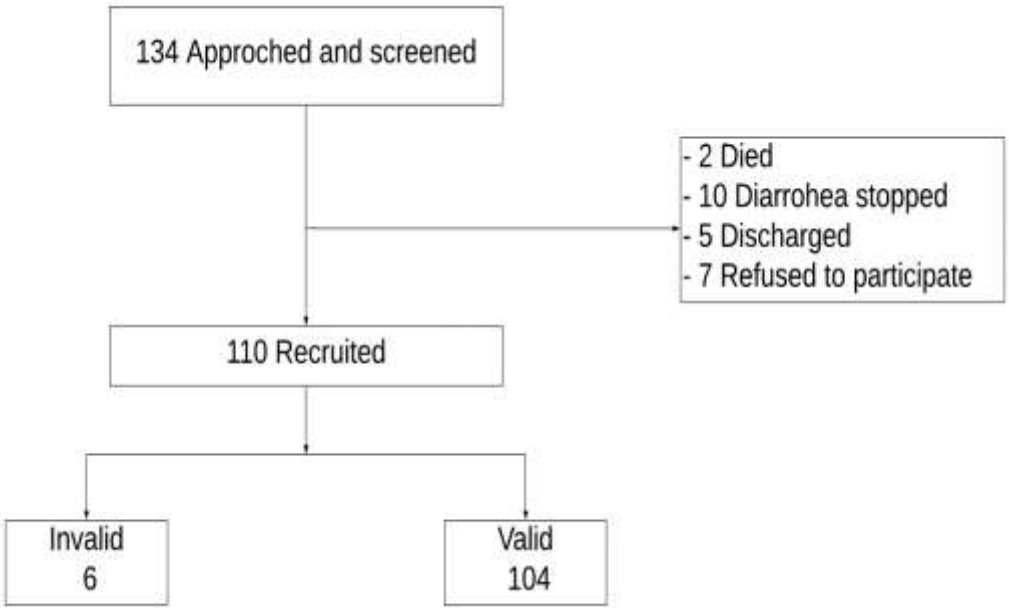

Figure 2: Enrolment schema of the study

4.1 Biodata of the study participants

The participants' age ranged from 18 to 87 years with a median age of 40.5 years (IQR 26). Majority (57.7 %) of the participants were female. Most (42.7%) of the participants used municipal water as their main source of drinking water. This is summarised in table 1 below.

Table 1: Biodata of the study participants

Biodata		**Frequency**	**Median (IQR); Percentage (%)**
Age (years) Median (IQR)		110	40.5 (26)
Gender	Male	49	44.6
	Female	61	55.4
Source of drinking water	Borehole	8	7.3
	Municipal	47	42.7
	Rainwater	6	5.5
	River	13	11.8
	Unreported	36	32.7

4.2 Clinical characteristics among the study participants

4.2.1 History of admissions among the study participants

Most (80.9%) of the participants in the study were recruited from the medical ward, whereas 19.1% were from surgical ward. The median duration of hospitalization was 7 days (IQR 13.5). Eighty percent of the study participants had one working diagnosis on admission while 20% had more than one working diagnosis. This is summarized in the Table 2 below.

Table 2: History of admissions among the study participants

4.2.1.1 First admitting diagnosis among the study participants

Clinical Characteristics		**Frequency**	**Median (IQR); Percentage (%)**
Duration of hospitalization (days) Median (IQR)		110	7 (13.5)
Admitting ward	Medical ward	89	80.9
	Surgical ward	21	19.1
Number of admitting diagnosis	1 diagnosis	88	80
	>1diagnosis	22	20

The most common first admitting diagnosis among the study participants was acute gastroenteritis (18.2%), lower limb injuries (11.8%) and pneumonia (10.9%). The remaining admitting diagnosis are summarized in table 3 below.

Table 3: First admitting diagnosis among the study participants

	Diagnosis	N (110)	Percentage (%)
First admitting diagnosis	Acute gastroenteritis	20	18.2
	Lower limb injuries	13	11.8
	Pneumonia	12	10.9
	Sepsis	9	8.2
	Meningitis	7	6.4
	Malignancy	6	5.5
	Septic ulcers	4	3.7
	Venous thromboembolism	4	3.7
	Acute kidney injury	3	2.8
	Acute febrile illness	2	1.8
	Congestive heart failure	2	1.8
	Upper limb injuries	2	1.8
	Acute hepatitis	2	1.8
	Malaria	2	1.8
	Neutropenic fevers	2	1.8
	Faecal impaction	2	1.8
	Peritonitis	2	1.8
	Anaemia	2	1.8
	Infective endocarditis	1	0.9
	Pericarditis	1	0.9
	Acute myocardial infarction	1	0.9
	Acute cholecystitis	1	0.9
	Cellulitis	1	0.9
	Chronic osteomyelitis	1	0.9
	Conversion disorder	1	0.9
	Cord compression	1	0.9
	Cerebrovascular accident	1	0.9
	Diabetic ketoacidosis	1	0.9
	Guillain Barre syndrome	1	0.9
	Obstructive jaundice? cause	1	0.9
	Peptic ulcer disease	1	0.9
	Vasculitis	1	0.9

4.2.1.2 Second and third admitting diagnosis among the study participants

Twenty percent of the study participants had more than one working diagnosis during the study recruitment with most of them presenting with acute kidney injury (45.4%) as their second admitting diagnosis, whereas two of the study participants had 3 working diagnosis during the study recruitment.

The table 4 below summarizes second and third admitting diagnosis.

Table 4: Second and third admitting diagnosis among the study participants

	Diagnosis	**N**	**Percentage (%)**
Second admitting diagnosis (22)	Acute kidney injury	10	45.4
	Tuberculosis	5	22.7
	Congestive cardiac failure	1	4.6
	Anaemia	1	4.6
	Diabetic ketoacidosis	1	4.6
	Threatened abortion	1	4.6
	Obstructive uropathy	1	4.6
	Oesophageal candidiasis	1	4.6
	Bipolar	1	4.6
Third admitting diagnosis (2)	Anaemia	1	50
	Pancreatitis	1	50

4.2.2 History of antibiotic use among the study participants

Of the 110 participants, 49.1% used 1 antibiotic and 50.9% used >1 antibiotic. Among 56 patients with the history of using more than 1 antibiotics, 75% had used 2 antibiotics and 25% had used 3 antibiotics before the onset of diarrhoea.

A majority of participants had a history of using cephalosporin before the onset of diarrhoea. Sixty percent of the participants had a history of using antibiotics for < 1 week before the onset of diarrhoea while 40% had a history of using for ≥ 1 week. The table 5 below shows a summary of the history of antibiotic use among the study participants and figure 3 below shows the classes of antibiotics used.

Table 5: History of antibiotic use among the study participants

Clinical characteristics		**N (110)**	**Percentage (%)**
Number of antibiotics used	1 Antibiotic used	54	49.1
	>1 Antibiotic used	56	50.9
Duration of antibiotic use	< 1 week	66	60
	≥ 1 week	44	40

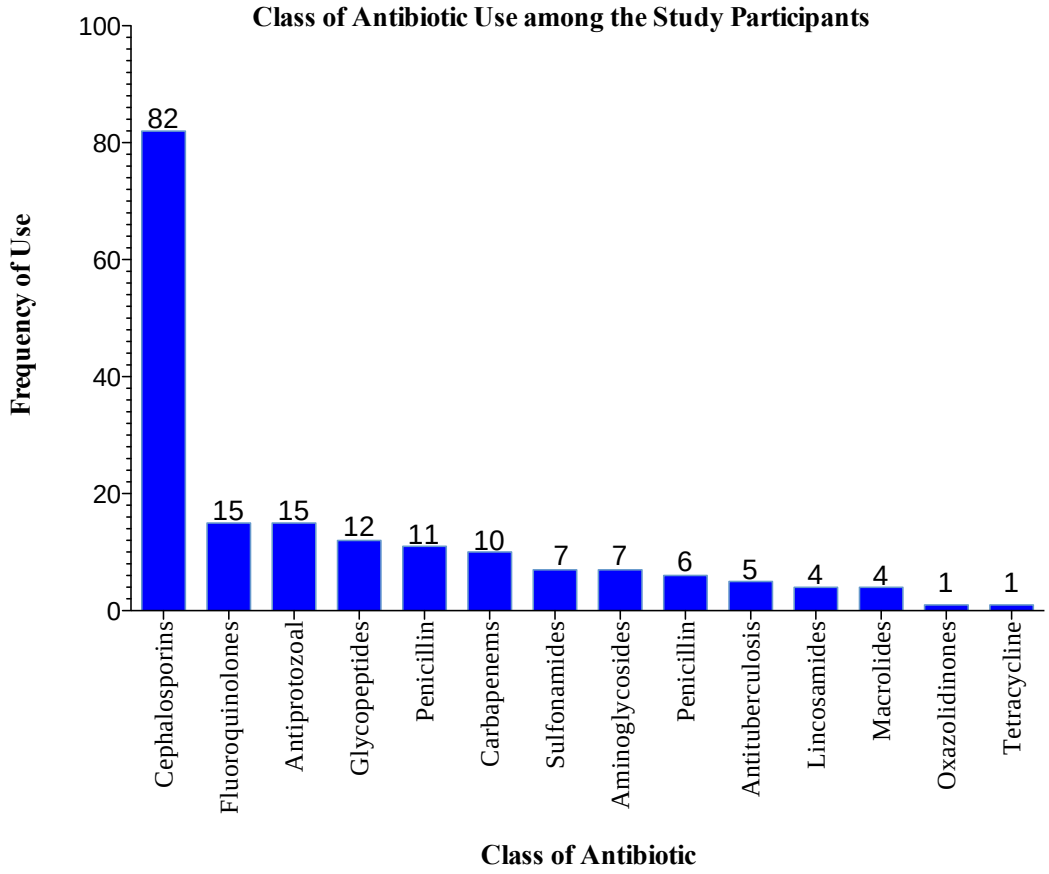

Figure 3: Class of antibiotic use among the study participants

4.2.3 History of gastric acid inhibitor use among the study participants

Participants who had a history of being on gastric acid inhibitor before the onset of diarrhoea were 41.8%, and 91.3% of participants on gastric acid inhibitor were on proton-pump inhibitors (PPIs). Most of the participants (52.2%), used the gastric acid inhibitors for < 1 week. Table 6 shows a summary of the history of gastric acid inhibitor use among study participants.

Table 6: History of gastric acid inhibitor use among the study participants

Clinical characteristics		N (110)	Percentage (%)
Usage of gastric acid inhibitor	No	64	58.2
	Yes	46	41.8
Type of gastric acid inhibitor used	PPIs	42	38.2
	H2RA	4	3.6
	None	64	58.2
Duration of gastric acid inhibitor use	< 1 week	24	52.2
	≥ 1 week	22	47.8

4.3.4 History of comorbidities among the study participants

Of the 110 participants recruited, 39.1% had a history of comorbidities. Of those with comorbidities 26.4% had a history of one comorbidity and 12.7% had a history of having more than one comorbidity during the study recruitment. The most common comorbidity among the participants was hypertension followed by diabetes mellitus and malignancy.

Table 7 summarises the history of comorbidities of the study participants and figure 4 gives a summary of the type of comorbidities seen among the study participants.

Table 7: History of comorbidities of the study participants

Clinical characteristics		N (110)	Percentage (%)
Comorbidities	No	67	60.9
	Yes	43	39.1
Number of comorbidities	No	67	60.9
	1 comorbidity	29	26.4
	>1 comorbidity	14	12.7

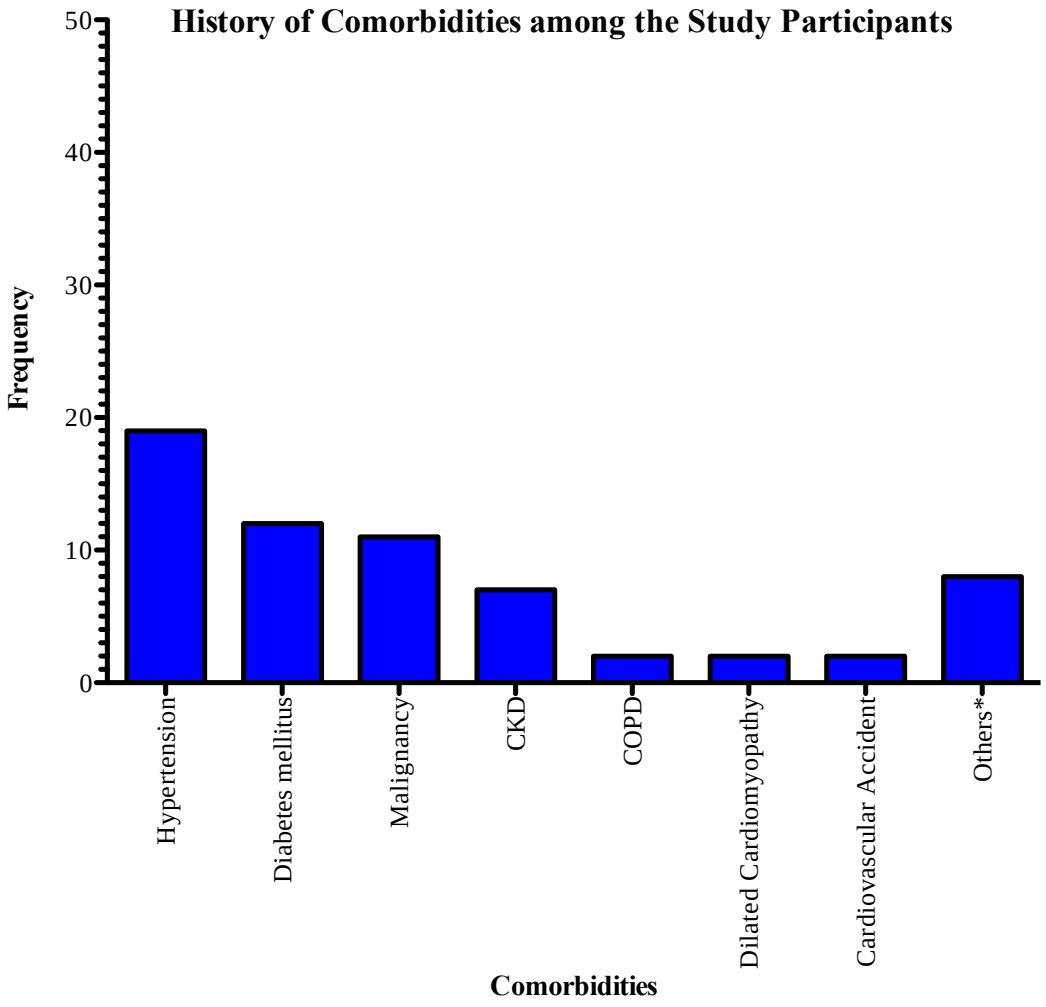

Figure 4: Type of comorbidities among the study participants

*Others: Rheumatic heart disease, Spinal injury, Polyarthritis, Systemic lupus erythematous, Coronary artery disease.

(CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease)

4.2.5 HIV status among the study participants

Most of the participants recruited (72.7%) were HIV negative as shown in table 8 below

Table 8: HIV status among the study participants

Clinical characteristics		N (110)	Percentage (%)
HIV	Negative	80	72.7
	Positive	30	27.8

4.3 Prevalence of *Clostridium difficile*-associated diarrhoea (CDAD)

All 110 stool specimens were tested by a molecular method (The Cepheid Xpert® *C. difficile/Epi* Assay) for rapid detection of toxin B gene sequences and 104/110 had valid and 6/110 had invalid results. Out of the 104 specimens with valid results, 23 had detectable (positive), 81 had undetectable (negative) gene sequences for *Clostridium difficile* toxin B. The assay further did a rapid detection for presumptive identification of, hypervirulent strain, toxigenic *Clostridium difficile* by checking for the presence of two of its characteristics: binary Toxin (*cdt*), and *tcdC* deletion 117 (*tcd*C□117), and it was not detected in any of the samples tested.

The prevalence of CDAD was 22.2% (95% CI 14.0- 30.2)

4.4 Comparison of biodata among participants with and without CDAD

The median age of the participants who had CDAD was 42 (IQR 22). Of the 104 participants the majority were female 60 (57.7%) and out of this 13 (21.7%) had CDAD.

Out of the 104 participants with valid results, 46 (67.6%) of them used municipal water as the source of drinking water and out of this 10 (21.7%) had CDAD.

There was no significant association between CDAD and the biodata of the study participants. The comparison of biodata of the study participants with and without CDAD is summarized in table 9 below.

Table 9: Comparison of biodata among study participants with and without CDAD

Participant characteristics		**Overall N (%)**	**CDAD + n (%)**	**CDAD – n (%)**	**p Value**
Age (years) median (IQR) (104)		39.5(25)	42(22)	39(26)	0.400[w]
Gender	Male	44(42.3%)	10(22.7%)	34(77.3%)	0.898[c]
	Female	60(57.7%)	13(21.7%)	47(78.3%)	
Source of drinking water (68)	Borehole	6(8.8%)	0(0%)	6(100.0%)	0.652 [f]
	Municipal	46(67.6%)	10(21.7%)	36(78.3%)	
	Rainwater	5(7.4%)	1(20%)	4(80%)	
	River	11(16.2%)	2(18.2 %)	9(81.8%)	

p value calculated by: [c]: chi-squared test, [w]: Wilcoxon rank-sum test, [f]: Fisher's exact test

4.5 Comparison of clinical characteristics among study participants with and without CDAD

4.5.1 Comparison of history of admissions among study participants with and without CDAD

Of the 104 participants, 84 (80.8%), were recruited from the medical ward, whereas 20 (19.2%) were from the surgical ward. Out of the 20, 9 (45%) had CDAD and this was statistically significant (p=0.01).

Of the 104 participants, 82 (78.8%) had one diagnosis during the admission. Out of the 82 (78.8%), 16 (19.5%) had CDAD and this was not statistically significant (p=0.22).

There was a statistically significant association of CDAD with duration of hospitalization (p=0.02). Participants with CDAD had a longer duration of hospitalisation: median (IQR) of 15 (7-25) days in comparison to those without CDAD who had a median (IQR) of 6 (4-14) days. Thus, participants who had a long hospital stay were more likely to get CDAD compared to participants who had a short stay. This data is summarised in table 10 below.

Table 10: Comparison of history of admissions among study participants with and without CDAD

Clinical characteristics		**Overall N=104**	**CDAD + n= 23**	**CDAD - n=81**	**p Value**
Admitting ward	Medical	84 (80.8%)	14 (16.7%)	70 (83.3%)	**0.010[c]**
	Surgical	20 (19.2%)	9 (45.0%)	11 (55.0%)	
Number of admitting diagnosis	1 diagnosis	82 (78.8%)	16 (19.5%)	66 (80.5%)	0.223[c]
	> 1 diagnosis	22 (21.2%)	7 (31.8%)	15 (68.2%)	
Duration of hospitalization – Days Median (IQR)		7(13.5)	15 (18)	6 (10)	**0.020[w]**

p value calculated by: [c]: chi-squared test, [w]: Wilcoxon rank-sum test

4.5.2 Comparison of history of antibiotic use among study participants with and without CDAD

There was a significant association (p=0.004) between participants with CDAD and history of using more than one antibiotic before the diarrhoea. Similarly, there was a significant relationship (p=0.03) with prolonged use of the antibiotics. Participants who had a history of using antibiotics for more than one week were more likely to have CDAD than participants who used for a shorter period. Most of the study participants were on cephalosporins as their first antibiotic. However, there was no statistical association between participants with CDAD and the class of antibiotic used. The above information is summarized in Table 11 and figure 5 below.

Table 11: Comparison of history of antibiotic use among study participants with and without CDAD

Clinical characteristics		Overall N=104	CDAD + n= 23	CDAD - n=81	p Value
Number of antibiotics used	1 Antibiotic used	50 (48.1%)	5 (10.0%)	45 (90.0%)	**0.004[c]**
	>1 Antibiotic used	54 (51.9%)	18 (33.3%)	36 (66.7%)	
Duration of antibiotic use	< 1 week	61 (58.7%)	9 (14.8%)	52 (85.2%)	**0.031[c]**
	≥ 1 week	43 (41.3%)	14 (32.6%)	29 (67.4%)	

p value calculated by: [c]: chi-squared test

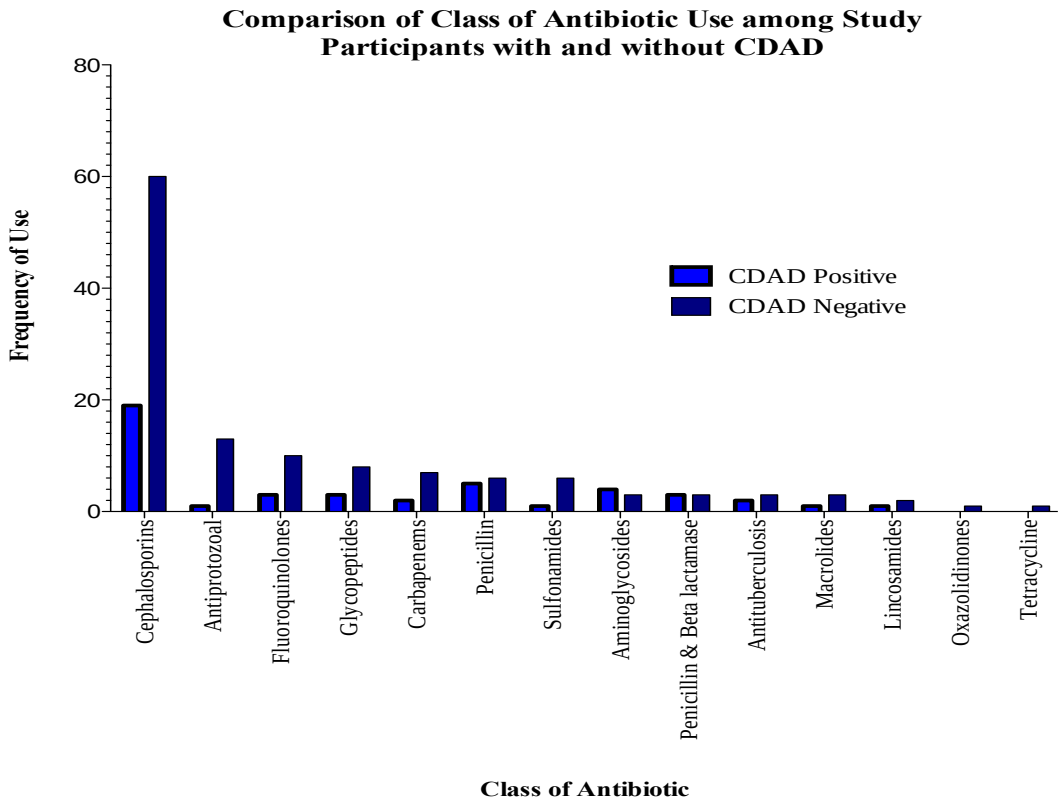

Figure 5: Comparison of the class of antibiotics used among study participants with and without CDAD

4.5.3 Comparison of history of gastric acid inhibitor use among study participants with and without CDAD

Of the 104 participants, 41.3% had a history of using gastric acid inhibitors before the onset of diarrhoea. Out of 23 (41.3%) CDAD was seen in 10 (23.3%) and this was not statistically significant association.

Of the 104 participants, 39 (37.5%) used PPIs as the gastric acid inhibitor, and 0ut of this 9 (23.1%) had CDAD. Most of the participants ,22 (51.2%), used gastric acid inhibitor for more than 1 week and CDAD was seen in 6 (27.3%).

There were no statistically significant differences between participants with CDAD and duration and type of gastric acid inhibitor used.

Table 12 below is a summary of comparison of history of use of gastric acid inhibitor among study participants with and without CDAD.

Table 12: Comparison of history of usage of gastric acid inhibitors among study participants with and without CDAD

Clinical characteristics		Overall N=104	CDAD + n= 23	CDAD - n=81	p value
Usage of gastric acid inhibitors	No	61(58.7%)	13 (21.3%)	48(78.7%)	0.814[c]
	Yes	43(41.3%)	10 (23.3%)	33(76.7%)	
Type of gastric acid inhibitors used	No	61(58.7%)	13(21.3%)	48(78.7%)	0.969[f]
	PPIs	39(37.5%)	9(23.1%)	30(76.9%)	
	H2RA	4(3.8%)	1(25%)	3(75%)	
Duration of gastric acid inhibitors used	< 1 week	21(48.8%)	4(19.0%))	17(81.0%)	0.523[f]
	≥ 1 week	22(51.2%)	6(27.3%)	16(72.7%)	

p value calculated by: [c]: chi-squared test, [f]: Fisher's exact test

4.5.4 Comparison of history of comorbidities and HIV among study participants with and without CDAD

Most of the study participants, 74 (71.2%), were HIV negative. Out of this 18 (24.3%) had CDAD. Out of 104 participants 40 (38.5%) had a history of having comorbidities and CDAD was seen in 10 (25%) .

Twenty six (25%) of the participants had a history of having one comorbidity, of which 5 (19.2%) had CDAD. There was no significant association between participants and the history of HIV and comorbidities. This information is summarised in table 13 below and figure 6 below summarises the comparison of comorbidities among study participants with and without CDAD.

Table 13: Comparison of history of Comorbidities and HIV among study participants with and without CDAD

Clinical characteristics		Overall N=104	CDAD + n= 23	CDAD - n=81	p value
HIV infected	Yes	30(28.8%)	5(16.7%)	25(83.3%)	0.394 [c]
	No	74(71.2%)	18(24.3%)	56(75.7%)	
History of comorbidities	Yes	40(38.5%)	10(25%)	30(75%)	0.575 [c]
	No	64(61.6%)	13(20.3%)	51(79.7%)	
Number of comorbidities	No	64(61.6%)	13(20.3%)	51(79.7%)	0.421 [c]
	1 comorbidity	26(25%)	5(19.2%)	21(80.8%)	
	>1 comorbidity	14(13.5%)	5(35.7%)	9(64.3%)	

p value calculated by: [c]: chi-squared test

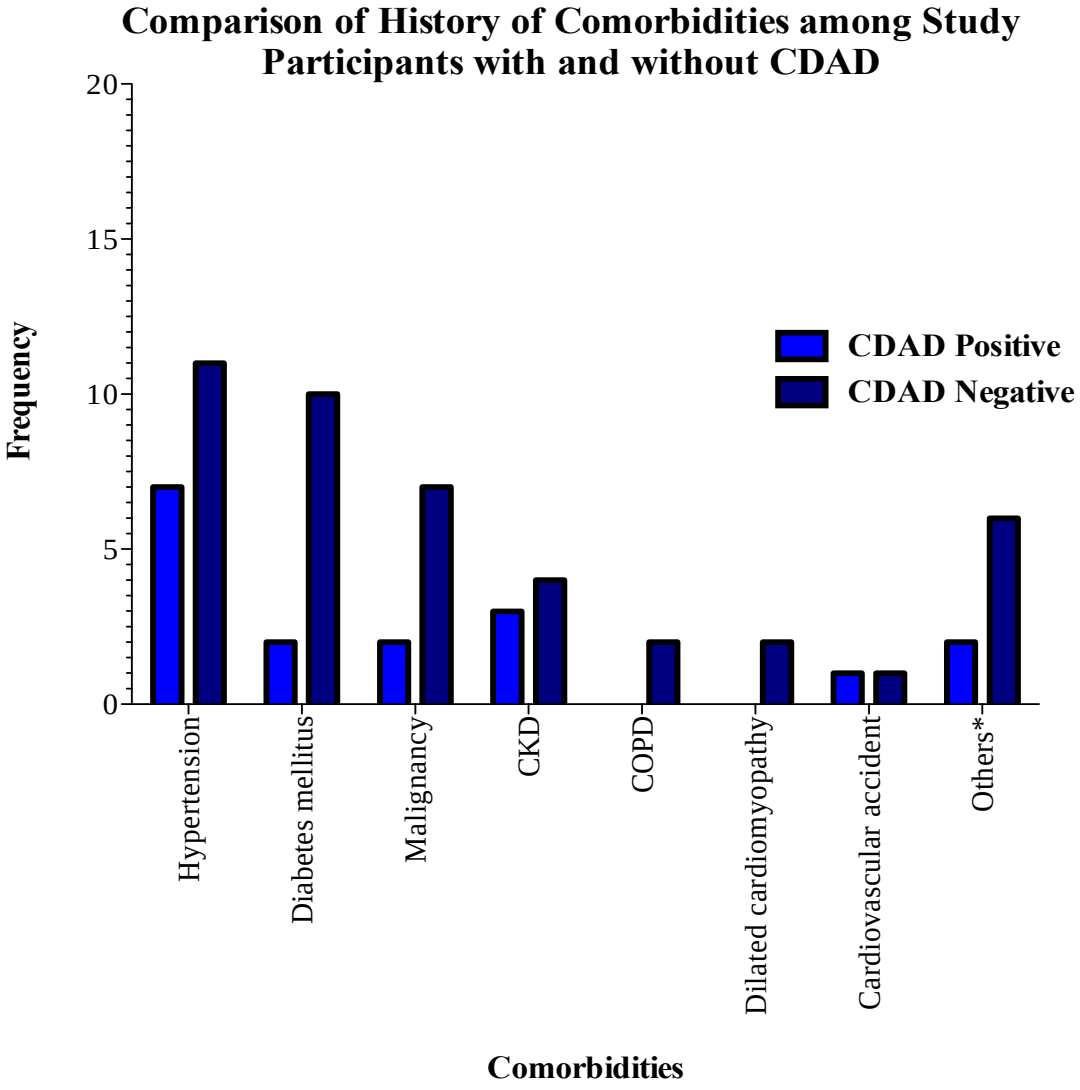

Figure 6: Comparison of comorbidities among study participants with and without CDAD

4.6 Factors associated with CDAD

Part of sub analysis of this data, a multivariate analysis was performed to test the level of association between various independent variables and CDAD as the dependent variable. Table 14 shows the adjusted odds ratio among study participants with CDAD. Although there was a wide confidence interval, usage of multiple antibiotics was noted to be significantly associated with CDAD.

Table 14: Adjusted odds ratio among study participants with CDAD

CDAD Positive	Adjusted Odds Ratio (95% CI)	Unadjusted Odds Ratio (95% CI)
Prolonged hospitalisation	2.62 (0.78-8.83)	4.28 (1.44-12.64)
Multiple antibiotic use	3.3 (1.03-10.01)	4.5 (1.52-13.30)
Prolonged duration of Antibiotic Use	1.68 (0.58-4.82)	2.79 (1.08-7.23)
Surgical Ward	0.72(0.34-1.49)	4.09 (1.43-11.71)

CHAPTER FIVE: DISCUSSION

5.1 Prevalence of *Clostridium difficile*-associated diarrhoea

Clostridium difficile is one of the leading health care associated infection in resource rich countries (Allegranzi & Pittet, 2009; Loo et al., 2005; Warny et al., 2005), and is considered as one of the major public health problems with significant medical and economic outcomes.

Scanty data achieved from the few studies done in Sub-Saharan Africa over the past two decades have found varied prevalence of *Clostridium difficile* infection/carriage ranging from 4.9% to 43% (Mwachari et al., 1998; Onwueme et al., 2011; Simango & Mwakurudza, 2008; Simango & Uladi, 2014). This study found a prevalence of 22.2% of CDAD, however no traces of the hypervirulent/ 027/NAP1/BI strain of *C. difficile* was isolated.

To the best of my knowledge, this is the first study in a tertiary referral hospital in Kenya, which used a molecular based method for isolation of *C. difficile* toxin from diarrhoeal stool specimen. Nucleic acid amplification test (NAAT) is not only a recommended diagnosis test by the latest IDSA guidelines (L Clifford McDonald et al., 2018), but is also found to be a superior test for detection of *C. difficile* toxins compared to the enzyme immunoassays (Chotiprasitsakul et al., 2012).

In 1998, a Kenyan study reported a prevalence of 0% but the study population was confined to HIV infected participants who presented with chronic diarrhoea (Mwachari et al., 1998). In addition, the Kenyan study used cell cytotoxic neutralisation assay, which has lower diagnostic accuracy when compared to NAAT. Furthermore, over the recent years, the global epidemiology of *Clostridium difficile*-associated diarrhoea has been constantly changing. For instance, in a retrospective

study done in Quebec, Canada, showed an incidence of CDAD increasing from 3-12 per 1000 persons (1991 to 2002) to 25-43 per 1000 persons (2003 to 2004) among hospitalised patients (Pépin et al., 2004). In another study, a population-based US study, they reported the overall incidence of nosocomial and community-acquired CDAD to have increased by 19.3-fold and 5.3-fold respectively (Khanna et al., 2012).

In contrast to Macharia et al's study (1998); but with stark similarity to this study, prevalence estimates from a South African study by Kullin et al (2015) approximate CDAD rates at 16% (Kullin et al., 2015)where NAAT testing was employed for diagnosis; nevertheless the author used a relatively less restrictive selection strategy where all participants with diarrhoea were tested for CDAD; whereas in this study only participants with diarrhoea and positive history of antibiotic exposure were selected. This might account for slightly higher prevalence rates reported in comparison to the South African study. Similar variations have been reported in South Africa and Malawi; both of which found a prevalence of 14% (Beadsworth et al., 2014; Samie et al., 2008).

Lower reported prevalence rates of CDAD seems to be a consistent trend across the sub Saharan African sub-continent. For instance, lower prevalence have been reported in Uganda (2.4%), Ghana (5%), Tanzania (6.4%) and Zimbabwe (8.6%) (Janssen et al., 2016; Khainza, 2014; Seugendo et al., 2015; Simango & Uladi, 2014). The disparity of the finding may be due to the different diagnostic test used. All these studies used immunoassays method to detect toxins rather than NAAT, which is a superior test (Chotiprasitsakul et al., 2012).

Moreover, the higher prevalence in this study may be related to the fact that there was an aggressive surveillance of history of recent antibiotic use. History of prior

antibiotic use is the most important and modifiable risk factor for CDAD (Hensgens et al., 2012; Loo et al., 2011).

In a recent meta-analysis, the pooled *Clostridium difficile* rate was 14.8% among all participants tested in Asia and it was 16.4% among hospitalized participants with diarrhoea (Borren et al., 2017). The prevalence findings in Asia are similar to other regions. Similar rates of CDAD, ranging from 7-20% were observed in a multicenter study by the Centers for Disease Control (CDC) surveillance in the United States (J. Cohen et al., 2013). Moreover, in a multi-country European surveillance study, they found that the proportion of CDAD to range from 4-39% (Bauer et al., 2011). These findings are comparable to the findings in this study.

A study in Nigeria by Onwueme et al. (2011), found the prevalence of CDAD among outpatient and inpatient participants to be 14% and 43% respectively (Onwueme et al., 2011). The prevalence among inpatients was much higher compared to the results of this study. This was most likely due to the fact that all the inpatient participants were HIV positives and were admitted for HIV related complications. This shows that most likely, the participants were severely immunocompromised and ill, whereby in this study participants were recruited irrespective of their HIV status.

The hypervirulent strain was not detected in any of this study samples. Among the few studies on CDAD in sub Saharan Africa, three studies looked at the profile of toxigenicity of the strains present in the stools sample. According to a study done in South Africa, they found 12/45 (26.7%) of CDAD positive participants had positive binary toxins and 2/45 (4.4%) had the hypervirulent strain (Samie et al., 2008). In a study done in Tanzania, 2 out of the 9 CDAD positive participants had binary toxins

positive and there was no hypervirulent strain of *C. difficile* was seen in that study (Seugendo et al., 2015). Consistent results were seen in Ghana (Janssen et al., 2016).

The findings on the hypervirulent strain in sub Saharan Africa are comparable to this study. These findings are similar to Asia where the prevalence of the hypervirulent strain was noted to be low compared to the prevalence's in resource rich countries (Borren et al., 2017; Davies et al., 2014). It has been noted that due to the guidelines applied by the infection control team, the prevalence of CDAD and the hypervirulent strain has been noted to be decreasing in Canada and United States (Giancola, Williams II, & Gentry, 2018; Katz et al., 2018).

The absence of the hypervirulent strain C. *difficile* clone in this study may explain the relatively benign clinical course among infected patients. Most of the participants responded well to metronidazole and were discharged to complete their treatment at home. In addition this study recruited a relatively younger population with a median (IQR) age of 42 (32-54), whereas in comparison; European studies which reported high prevalence estimates of the hypervirulent strain had a much older population aged ≥65 years (Bauer et al., 2011).

5.2 Biodata of the participants with CDAD

There was a slight female predominance (56.5%) in this study. Three other studies that were comparable to this study were studies done by Samie et al (2008), Rajabally et al (2013) and Kullin et al (2015)that showed female to have a predominance of 56.5%, 58%, and 57.6% respectively (Kullin et al., 2015; Rajabally et al., 2013; Samie et al., 2008). Despite female participants having a slight predominance over male, none of these studies showed gender being statistically significant in participants having CDAD.

In this study, participants who had CDAD had a median (IQR) age of 42 (32-54), which was consistent with the South African study by Rajabally et al (2013), whereby the median (IQR) age was 41 (31-55) (Rajabally et al., 2013). In both studies age was not statistically significant. The results of other studies done in Sub Saharan Africa, for instance studies done in Zimbabwe, Tanzania and Nigeria were consistent with findings of this study except a study that was done in Ghana that showed that there was an association between CDAD positive participants with children < 5 years of age (Janssen et al., 2016). In contrast, other studies have showed that advanced age is a risk factor for CDAD (Dale N Gerding, 1997; Loo et al., 2011; Pepin, Saheb, et al., 2005). The differences with this study might be explained by age differences; this study had a younger population compared to the studies in resource rich countries.

Similarly, there was no relationship between CDAD positive participants with source of drinking water (p=0.65). To date there has been no study which showed relationship between CDAD with source of drinking water.

5.3 Clinical characteristics of the participants with CDAD

In this study, the median duration of hospitalization for participants with CDAD was 15 days (IQR:18). This result is comparable to a study done in New York by Stevens V et al (2011), who found the median duration of hospitalization was 12 days (IQR:17) (Stevens et al., 2011). There was a statistically significant association of CDAD positive with duration of hospitalization (p=0.02) in both studies. This finding was consistent with data from previous studies that identified that prolonged duration of hospitalization is a risk factor for CDAD (Dale N Gerding, 1997; Pepin, Saheb, et al., 2005; Seugendo et al., 2015). The increase in chances of getting CDAD during hospitalization may be a surrogate for the duration and degree of exposure to the organism.

This study showed that there was an increased risk of CDAD in participants with increasing number, and days of antibiotic exposure. Those participants on more than one antibiotic (p=0.004), and those on antibiotics for more than one week (p=0.031) were at greater risk of having CDAD than those who are on a single antibiotic and less than one week exposure to antibiotics. This is in accordance with findings reported in the previous studies (Dale N Gerding, 1997; Loo et al., 2005; Pepin, Saheb, et al., 2005). Unnecessary use of empiric antibiotics and prolonged treatment should be discouraged.

The exposure to multiple antibiotics and longer exposure to antibiotics all result in a remarkable decrease of normal flora relative to using a single antibiotic and shorter exposure to antibiotics. Thereby, extending the participants window of susceptibility to subsequent CDAD.

Even though the majority of the participants were on cephalosporins, there was no significant association of the class of antibiotic and participants with CDAD. The findings of this study differ from other studies that have shown that usage of any antibiotic is basically associated with CDAD, although some classes of antibiotics like cephalosporins, quinolone, clindamycin and broad-spectrum penicillins have been found to have a higher risk (Janssen et al., 2016; Loo et al., 2005; Pepin, Saheb, et al., 2005; Stevens et al., 2011). This study may have been underpowered to detect an association.

The data reported here showed no association between CDAD and having more than one admitting diagnosis during admission. Being admitted to the surgical ward was one of the risk factors for CDAD (p=0.01) in this study. In previous studies, admission to the surgical wards was a risk factor. This was not really the ward per se

that was the risk factor but rather the antibiotic exposure in that ward (Brown, Valenta, Fisman, Simor, & Daneman, 2015).

Whether use of gastric acid inhibitors is a risk factor for CDAD or not is still controversial. In spite of the fact that there are various studies that suggest an epidemiologic association between CDAD and usage of gastric acid inhibitors (Cunningham et al., 2003; Dial et al., 2004; Janarthanan et al., 2012; Stevens et al., 2011), outcomes from other well-controlled studies show that the association is the result of confounding with advanced age, duration of hospitalization and the underlying severity of illness (Loo et al., 2005; Novack et al., 2014; Pepin, Saheb, et al., 2005; Weiss et al., 2015).

There was no significant increase risk of CDAD with the use of gastric acid inhibitors in this study and this results are consistent with the South African study by Rajabally et al (Rajabally et al., 2013).

Kenya is a high HIV-prevalent country, yet CDAD was not associated with HIV infection in this study. This results are comparable to 2 different studies done in South Africa by Samie et al and Rajabally et al, and a study that was done in Malawi and Ghana (Beadsworth et al., 2014; Janssen et al., 2016; Rajabally et al., 2013; Samie et al., 2008). This study may have been underpowered to detect this association. In addition, HIV infected participants were not characterized in terms of HIV disease state and CD4 count, thus it is possible that participants were not severely immunosuppressed.

However, studies done in Nigeria and Tanzania showed that the association between CDAD and HIV infection was statistically significant (Onwueme et al., 2011; Seugendo et al., 2015). Available data have showed that one of the important causes

of bacterial diarrhoea among HIV infected patients in USA is *C. difficile*. It has been proposed that HIV infected patients are at increased risk of getting CDAD. Since they are having an underlying immunosuppression, they are more likely to be exposed to healthcare facilities, exposed to antibiotics or a combination of these factors (Sanchez et al., 2005).

In previous studies, the severity of underlying disease and history of comorbidity was a risk factor for developing CDAD (Changela et al., 2004; L. McFarland, Surawicz, & Stamm, 1991). However, these studies did not adjust for duration of hospitalization and age. This study did not establish an association between CDAD and history of comorbidities. This could have been because of the few participants who had a history of comorbidity.

Previously, most of the patients admitted in this hospital were put on antibiotic. Upon developing diarrhoea in the wards, the diarrhoea was not approached as CDAD. However, by creating awareness of the high prevalence of CDAD in our setup, the clinicians will shorten the duration of antibiotic use and minimise the use of multiple antibiotics, unless justified, hence lowering the prevalence. However, the findings of this study are hypothesis generating to open up the need of other studies to further elaborate on the risk factors associated with CDAD and to investigate on ways of lowering the prevalence. In addition to that, other studies can check on the outcomes of CDAD in this setup.

5.4 Summary of the key findings

In summary, there is a moderately high prevalence (22.2%) of CDAD among patients at Moi Teaching & Referral Hospital, a tertiary hospital serving mostly North Rift region of Kenya. From this study finding, it's possible that duration of hospitalization,

exposure to longer duration of antibiotic use or exposure to multiple antibiotic use attribute additional risk to CDAD; however, the exact nature of the relationship in terms of cause and effect within the MTRH context will need to be ascertained.

5.5 Study strengths and limitations

The strength of this study was the use of PCR as a diagnostic tool to detect the presence of toxin B. PCR is the most sensitive diagnostic test, currently recommended by the IDSA latest guidelines (L Clifford McDonald et al., 2018).

This study had certain limitations. Since the diagnostic test was only checking on the presence of toxin B, there is a possibility we may have underestimated the proportion of participants who had CDAD secondary to positive toxin A. Moreover, molecular typing of *C. difficile* strains was not done to determine the different ribotypes of strains present in our setup. Lastly, cause and effect could not be established between CDAD and identified risk factors given the cross-sectional nature of the study.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. There was moderately high prevalence (22.2%) of *Clostridium difficile*-associated diarrhoea (CDAD) in Moi Teaching and Referral Hospital.
2. The possible risk factors for CDAD in this setup include: long duration of hospitalization, usage of multiple antibiotics and prolonged duration of antibiotic exposure.

6.2 Recommendations

1. To test for CDAD in anyone admitted with history of long duration of hospitalisation, history of multiple or longer exposure to antibiotics.
2. Having a routine diagnostic service for detection of *C. difficile* toxins in MTRH laboratory, as early detection has been associated with reduced morbidity and mortality.
3. A prospective study to be done to determine risk factors and outcomes of CDAD in this setup.

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APPENDICES

Appendix I: Consent for participation (English)

My name is Dr. Amal Salim. I am a qualified doctor, registered by the Kenya Medical Practitioners and Dentists Board. I am currently pursuing a Masters degree in Department of Internal Medicine at Moi University School of Medicine. I would like to recruit you into my research which is to study on the prevalence of *Clostridium difficile*-associated Diarrhoea in Moi Teaching and Referral Hospital.

The study shall involve an initial interview of about 5 minutes. Subsequently you shall have your stool taken for a test that enables us to identify *Clostridium difficile* strains using the molecular method Xpert® *C. difficile* (Cepheid,GX CDifficile-CE-10). The test will be free of charge.

The benefit of participating in this study to you as an individual is to get to know your health status as regards the diagnosis of having *Clostridium difficile*-associated Diarrhoea. A copy of your results will be shared with clinical team taking care of you. The decision about your treatment will be made by the team.

This study is of minimal risk. The benefits of this study outweigh its risks.

The results of the study will be stored in a database that is password protected and only accessible by those conducting the study. No one will be able to identify you or your results. Should the data be published, no individual information will be disclosed. Your participation in this study is voluntary. If you decide to participate, you can change your mind later and quit the study before the end of the study. If you decide not to participate, or if you quit the study, it will not affect the health care services you receive in the wards. By signing this document, you are voluntarily

agreeing to participate. You are free to decline to answer any particular question you do not wish to answer for any reason.

This study has been approved by the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital.

If you need further clarifications please contact IREC using the address below:

The Chairman IREC,

Moi Teaching and Referral Hospital,

PO Box 3,

Eldoret.

Tel: 05333471/2/3

My cell phone number is: 0721 718 037.

I have read this informed consent and authorization form. ALL OF MY QUESTIONS HAVE BEEN SATISFACTORILY ANSWERED, AND I WANT TO TAKE PART IN THIS RESEARCH STUDY. By signing below, I give my permission to participate in this research study and for the described uses and releases of information.

Sign: …….

Name: …….

Date: …….

Appendix II: Consent for participation (Swahili)

IDHINI YA KUHUSISHWA

Jina langu ni Daktari Amal Salim. Mimi ni daktari niliyesajiliwa na bodi ya madaktari wa Kenya (Kenya Medical Practitioners and Dentists Board). Mimi ni msomi wa shahada ya juu (Masters) ya udaktari (Internal medicine)katika chuo kikuu cha Moi. Ningependelea uhusike katika utafiti unaohusu kiwango cha maambukizi ya kimelea *Clostridium difficile* kati ya wagonjwa wanaoharisha katika hospitaliya Rufaa na Mafunzo ya Moi.

Uchunguzi huu utahusisha kuulizwa maswali katika dakika 5 zaawali. Kisha, kinyesi chako kitachukuliwa na kupimwa kimelea *Clostridium difficile* kutumia mbinu ya Xpert® *C. difficile*(Cepheid, GX CDifficile-CE-10). Utafiti huu hautakugharimu malipo yoyote.

Faida ya kushiriki katika utafiti huu, kwa wewe binafsi, ni kupata kujua hali ya afya yako inayohusiana wa utambuzi wa kama chanzo cha kuharisha ni kimelea *Clostridium difficile*.Nakala ya matokeo yako itapewa timu ya madaktari wenye kukuhudumikia. Timu ya madakatari wako ndio wataamua kuhusu matibabu yako.

Utafiti huu ni wa hatari ndogo. Faida ya utafiti huu unashinda hatari yake.

Matokeo ya utafiti huu yatawekwa kodi ya siri kwenye orodha iliyo na ulinzi kamilifu na ambao inaweza kufiki wanawatafiti pekee yao. Hakuna yeyote mwingine atakayeweza kufikia matokeo ya vipimo yako. Ikiwa matokeo yatachapishwa, habari zozote za kibinafsi hazitachapishwa.Uhusishwaji wako kwenye utafiti huu ni wahiari. Baada ya kushiriki utafiti, unawezakubadili nia nakujitoa kabla ya utafiti kumalizika.Kutokushiriki au kujiitoa kwenye utafiti, haita athiri matibabu utakayopewa katika hospitali yetu. Kwa kutia sahihi hati hii unatoa idhini ya

kushirikishwa kwa hiari yako, pia unauhuru wakutojibu swali lolote unalotaka,kwa sababu yoyote ile.

Uchunguzi huu umeidhinishwa na KamatiyaMaadiliyaTathmininaUtafitiya Chuo Kikuu cha MoinaHospitaliyaRufaanaMafunzoyaMoi.

Iwapo unahitaji maelezo zaidi tafadhali wasiliana na IREC kwa kutumia anwani ifuatayo:

Mwenyekiti IREC,

Moi Teaching and Referral Hospital,

S. L. P.3,

Eldoret.

Simu: 05333471/2/3

Nambari yangu ya simu ya rununu ni: 0721 718 037

Nimesoma nakuelewa hati hii ya idhini ya hiari nakutoaradhi. MASWALI YANGU YOTE YAMEJIBIWA KWA NJIA INAYORIDHISHA NA NINGEPENDELEA KUHUSIKA KATIKA UTAFITI HUU. Kwa kutia sahihi hapa, nimetoa idhini kuhusishwa kwenye utafiti huu pamoja nakutumiwa nakuchapishwa kwa matokeo yake.

Sign: …….

Name: …….

Date: …….

Appendix III: Data collection form

Date: ……..

Age: ……...

Gender: ……..

Admitting Diagnosis: ……

Admitted Ward Medical ward ☐ ICU ☐ CCU ☐

Surgical ward ☐ Gynaecology ward ☐

History of Co-morbidities (DM, HTN, cardiac disease, renal disease)

……..

HIV Status Positive ☐ Negative ☐

Duration of Hospital Stay…….

Class of Antibiotic used……

Duration of Antibiotic use……

Usage of Gastric Acid Inhibitor: YES ☐ NO ☐

If yes, Type of Gastric Acid Inhibitor: …….

Duration of Gastric Acid Inhibitor use: ……...

Source of Drinking Water……

Appendix IV: Procedure for checking on the *C. difficile* toxins

Specimen sampling and handling:

1. Collect the stool specimen in a dry, clean container.
2. Label the container with Sample ID.
3. Place the container in a sealed plastic bag to avoid contamination.
4. Transfer the sample to the AMPATH Reference laboratory where a rapid, automated *in vitro* diagnostic test, real-time polymerase chain reaction (PCR) (Cepheid Xpert C. difficile/Epi, Cepheid, Sunnyvale CA), to identify toxin-producing *C. difficile* strains, toxin B and presumptive identification of NAP1/B1/027 strain will be done.
5. The specimen is stable for up to 5 days when stored at 2–8°C. Alternatively, specimens can be kept at room temperature (20-30°C) for up to 24 hours.

Device Description

The Cepheid Xpert® *C. difficile/Epi* Assay is a multiplex real-time PCR that detects the toxin B gene (tcdB), the binary toxin gene (cdt), and the tcdC gene deletion at nt117. The extraction, amplification, and detection steps take place in different chambers of a self-contained, single-use cartridge containing all the reagents necessary for the detection of *C. difficile* gene targets. The assay is performed on the Cepheid GeneXpert® Dx System.

Procedure of Preparing the Cartridge

1. Remove the Xpert *C. difficile* cartridge and sample reagent from the package to equilibrate to room temperature (approximately 15 minutes)
2. Immerse a sterile swab into a stool specimen, ensuring that the swab is ¾ and lightly covered with the stool specimen.
3. Insert the swab into the reagent vial.
4. Break the swab into the vial ensuring that swab is short enough to allow the cap to close tightly
5. Vortex for 10 seconds.
6. Open the cartridge lid and using a transfer pipette (supplied in the kit) add the entire contents of the sample reagent vial to the cartridge "S"chamber.
7. Close the cartridge lid and load into the Gene Xpert Infinity as per Process/Order Test, and the test was performed using the Gene Xpert *C. difficile* assay program.

Figure 3 below shows Cepheid Xpert *C.difficile* kit and Cepheid GeneXpert® Dx System.

Figure 7: Cepheid Xpert *C.difficile* kit and Cepheid GeneXpert® Dx System

Potential results included the following: toxigenic *C. difficile* positive/presumptive 027-NAP1-BI negative, toxigenic *C. difficile* positive/presumptive 027-NAP1-BI

positive, toxigenic *C. difficile* negative/presumptive 027-NAP1-BI negative, invalid, error, or no results.

The figure 4 below shows some of the possible results that can be seen after the test.

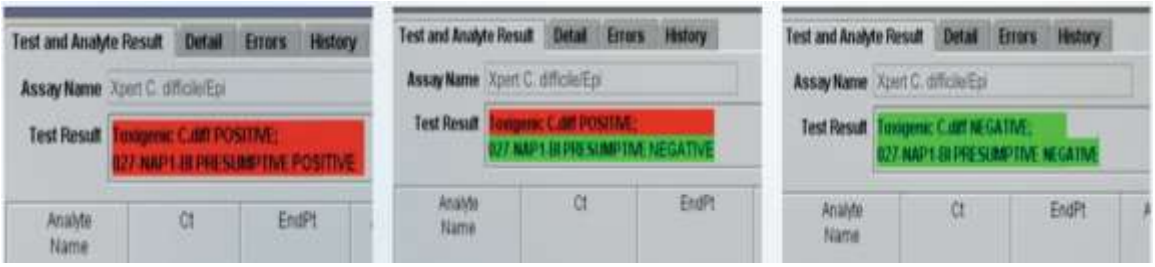


Figure 8: Possible Cepheid Xpert® *C. difficile/Epi* assay results

Principle of the Procedure

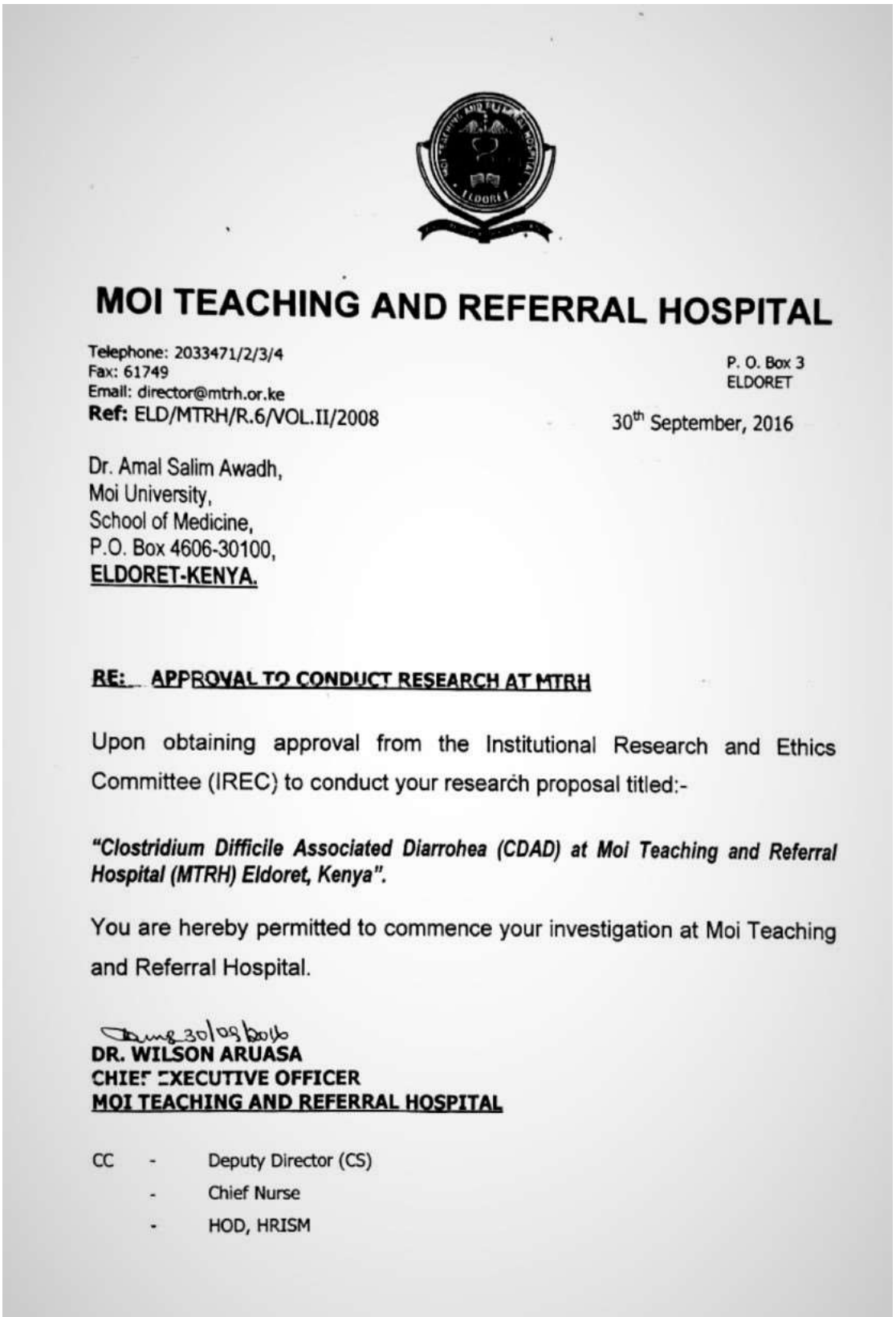
The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR assays.

The primers and probes in the Xpert *C. difficile* assay detect sequences in the genes for Toxin B (*tcdB*), Binary Toxin (*cdt*), and *tcdC* deletion 117 (*tcd*C□117).

Xpert *C. difficile* Assay includes reagents for the detection of Toxin producing *C. difficile* and Toxin producing *C. difficile*, presumptive 027/NAP1/BI respectively as well as the Sample –Processing Control (SPC). The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Sensitivity and Specificity of the Xpert *C. difficile* is 93.5% and 94.0% respectively.

Appendix V: IREC and MTRH approval letter



MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

30th September, 2016

Dr. Amal Salim Awadh,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Clostridium Difficile Associated Diarrohea (CDAD) at Moi Teaching and Referral Hospital (MTRH) Eldoret, Kenya".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

30/08/2016

DR. WILSON ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/184
Approval Number: 0001759

27th September, 2016

Dr. Amal Salim Awadh,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 SEP 2016
APPROVED
P.O. Box 4606-30100 ELDORET

Dear Dr. Amal,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Clostridium Difficile Associated Diarrohea (CDAD) at Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya".

Your proposal has been granted a Formal Approval Number ***FAN: IREC 1759*** on 27th September, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th September, 2017. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

For

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD