Isolation and characterization of toxigenic *Clostridium difficile* in selected outpatient Health Facilities within Nairobi County

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W64/8348/2017

A dissertation submitted in partial fulfillment of the requirements for the award of the Master of Science degree in Tropical and Infectious Diseases.

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DECLARATION OF ORIGINALITY

I, duly proclaim that this is my original work and has not to the best of my knowledge been presented anywhere else in any institution of higher learning for the award of any degree.

I, totally understand that any false claim in respect of this work shall result in disciplinary action, in accordance with the University Plagiarism Policy

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DEDICATION

This dissertation is dedicated to my immediate family; Martin, Alvin, and Devina for their endless moral support.

My parents, Mr. and Mrs. Maina for believing in my dreams in the bleak of scarcity.

Above all the grace of God has brought me this far.

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ACRONYMS

H$_2$RAs   Histamine receptor antagonists

NSAIDs Non-steroidal anti-inflammatory drugs

TcdA   Toxin A

TcdB   Toxin B

PCR   Polymerase Chain Reaction

PPI   Proton Pump Inhibitors

CDI   *Clostridium difficile* Infection

CACDI   Community-acquired Clostridium difficile infection

HACDI   Hospital-acquired Clostridium difficile infection

CDAD   *Clostridium difficile* associated disease

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background

In the last four decades, the spectrum encompassing the epidemiology and management of *Clostridium difficile* associated diseases (CDAD) has changed progressively. These changes resonate with heightened rates of unprecedented incidences, morbidity and mortality of novel community-acquired *Clostridium difficile* infections with severity index matching superbugs in fatal nosocomial infections and multi-drug resistance. Significant risk factors in CACDI are indefinite and challenging primarily on pathological relevance of toxigenic and non-toxigenic strains, pathways of transmission, growing antimicrobial resistance and treatment failure hypothesized on the emergence of epidemic strains and other independent risk factors. *Clostridium difficile* has gained public health significance in the community settings necessitating surveillance and monitoring the prevalence, risk factors and strains responsible for this.

Objective

To evaluate the prevalence, the risk factors of community-acquired *Clostridium difficile* infection and profile the toxin genes of the recovered isolates.

Methodology

This was a cross-sectional prospective study conducted between August and November 2019 at the Mama Lucy Kibaki and Mbagathi referral and teaching hospitals within Nairobi County. From a total of 342 diarrhoeal samples, 301 were processed and analyzed. Subsequently, clinical and socio-demographic data was collected at the time of the illness using a guided structured questionnaire. Growth on CHROMagar was assessed for colonial morphology by ultraviolet light fluorescence and gram staining. DNA extraction was done using the Meridian Bioscience ISOLATE II Genomic DNA Kit following the manufacturer's instructions, after which the *tcdA, tcdB* and the binary toxin *CDTa/b* genes were detected by multiplex conventional PCR. Data was entered into excel spreadsheets and exported for statistical analysis into the IBM SPSS statistics Version 20.

Results

In all 301 samples tested, 36 (12.0%) were culture positive for *Clostridium difficile* and 35 (11.6%) were positive for toxins genes. Mixed enteropathogens and parasites were not characterized. Age of participants varied from 1-62 years, and mean age was 27.2±15.7, with more than half of the study participants being females 50.6. The patients presented with bloating 36%, cramping 33%, bloody 13%, and 6% of fever, headache and vomiting. Among the 36 (12.0%) CACDI positive, 58.3% were males, mean age 24.9 ±13.7 years. Potential risk factors for CACDI were use of NSAIDs (p=0.004), HIV/AIDs as a comorbidity (p=0.001), history of organ transplant (p=0.04), keeping a pet or a farm animal (p=0.03) and the use of acid suppressants (p=0.005). Symptoms in admixture of diarrhoea that were telltale signs for CDI were gastrointestinal bleeding presenting as bloody stool P=0.003), abdominal cramping (p=0.002). The toxins profile observed included *tcdA+/tcdB+/CDTa-/b-* 18 (50%), *tcdA-/tcdB+/CDTa+/b+* 9 (25%)*, tcdA-/tcdB-/CDTa-/b-* 1 (3%) and *tcdA-/tcdB+/CDTa+/b+* 3 (8%) and *tcdA+/tcdB+/CTDa+/b+* 5 (14%).

Conclusion

CACDI seems to occur across all age groups as the risk factors keep evolving yet the public health impact largely remains incompletely defined especially in developing countries calling for active surveillance, awareness, early and routine testing and containment strategies.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Clostridium difficile comes from the Greek word "Kloster" meaning spindle and belongs to the family *Clostridiaceae* (HALL & O'TOOLE, 1935). It is a toxin-producing, gram-positive, motile, sporulating, obligate anaerobic bacillus (Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, 2016). Primarily, it is spread by the fecal-oral route (Sayedy et al., 2010; Durovic & Widmer, 2018). It is an enteric commensal in 5-15% of non-diseased adults, roughly accounts for 30-35% in newborns,10-15% in infancy (Liao et al., 2018) and about 57% in long term care health facilities residents (Surawicz et al., 2013) (Hung et al., 2015) (Kazanowski, Smolarek, Kinnarney, & Grzebieniak, 2014) (Goudarzi, Seyedjavadi, Goudarzi, Mehdizadeh Aghdam, & Nazeri, 2014).In 1935 Hall and O'Toole first called it *Bacillus difficilis* owing to difficulties in isolation and characterization. Contrastingly, it was non-toxigenic in the stool of healthy infants but toxigenic in guinea pigs thus renamed in the 1970s (HALL & O'TOOLE, 1935). Henceforth, it is classified as a causative pathogen of infectious diarrhoea due to the irrational use of antibiotics (Sahil Khanna & Pardi, 2010), contributory to the distortion of its niche as an enteric commensal hence the crossover to an enteric pathogen. *Clostridium difficile* associated disease (CDAD) previously, largely classified a nosocomial infection is now in the community. It can be self-limiting diarrhoea or an aggravated disease. The latter presents with perforation and bleeding in the gastrointestinal tract with eventual septic shock and death. In severity it's marked by "Pseudomembranous colitis" and "toxic megacolon" (Dapa & Unnikrishnan, 2013; Manek et al., 2011; Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, 2016).

Globally, CDAD is a public health challenge (Eze, Balsells, Kyaw, & Nair, 2017), colonizing both animal and human populations (Rodriguez, Taminiau, Broeck, Delme, & Daube, 2016). Lately, clinical isolates have shown species overlap upon molecular characterization; like the "ribotype (RT) 078" (Rabold et al., 2018). The undiagnosed cases in the community can be attributed to scarcity of knowledge in epidemiology (Chitnis et al., 2013), transmission and non- routine diagnosis (Wilcox, 2012; Lim et al., 2014) yet *Clostridium difficile* remains pathogenic in settings devoid of the renowned risk factors (Sahil Khanna et al., 2012) especially now when the

community settings have been recognized as potential reservoir(s) of infection among healthy individuals, in food, in water and in animals (S Khanna, Pardi, Aronson, Kammer, & Baddour, 2012; Bloomfield & Riley, 2016). *Clostridium difficile* is progressively a causative agent of diarrhoea in the community (Suárez-Bode, Barrón, Pérez, & Mena, 2019). The burden of CACDI as observed by Maisa et al has increased (Maisa et al., 2019).

Over the last three decades, there has been the emergence of novel strains of CD (Jassem et al., 2016). Findings by Freeman et al exemplify the inadequate knowledge on the genesis of the factors stirring the rising incidence and prevalence of *Clostridium difficile* infection (CDI), the evolving scope of new risk factors and the unconventional clinical presentation (Freeman et al., 2010), especially with community acquired *Clostridium difficile* infection (CACDI).

It has been opined that the changing epidemiological landscape of *Clostridium difficile* infection (CDI) is related to the evolution of complementary virulent genes on the novel strains alongside their specific exotoxin(s) (Goudarzi et al., 2014). This realization comes with an awareness of an associated surge in ease of transmission, relapse rates and failed therapy (Ofori et al., 2018). *Clostridium difficile* has exhibited genetic variants (Gupta & Khanna, 2014) with over 150 PCR ribotypes and 24 toxin variant strains (Kuijper, Coignard, & Tüll, 2006) serving to emphasize the hypothesized mounting virulence of *Clostridium difficile*, the change in epidemiology and overall treatment outcomes of CDI.

Recent studies approximate that 50% of all CDI cases have a community origin and an incidence rate estimated at 25- 30 per 100,000 populations (Sahil Khanna et al., 2012), a further ~24% of the patients with no history of antimicrobials use before the onset of the CDI (Khanna et al., 2012). Furthermore, 40% of all CACDI require hospitalization where severity has been linked to the "hypervirulent ribotypes" 027 and 078 and further necessitated a modification of the treatment guidelines (Ofori et al., 2018; McDonald et al., 2018) owing to the development of resistance to broad spectrum of antibiotics, more so the recommended first line regimen of vancomycin and metronidazole (Ngernsombat, Sreesai, Harnvoravongchai, Chankhamhaengdecha, & Janvilisri, 2017). Antimicrobial resistance is a factor in the changing epidemiology of CDAD (Spigaglia, Mastrantonio, & Barbanti, 2018) associated severity and observed recurrence rates (Harnvoravongchai, Pipatthana, Chankhamhaengdecha, & Janvilisri, 2017). Clinical isolates exhibiting multi drug resistance (MDR) (Ngernsombat et al., 2017) further epitomize the factors

driving the evolving epidemiological scope of CDAD. Not to mention, the ability of *Clostridium difficile* to adapt to new environmental conditions (Thiyagarajan & Gorayan, 2017) enhancing the occurrence outside the scope of conventional risk factors (Chitnis et al., 2013).

A recent study by Chitnis et al show that a prior exposure to antibiotics before the onset of disease may necessarily not be a risk factor for CACDI (Chitnis et al., 2013). Khanna et al found that CACDI is occurring in populations that are younger, mostly females, with no co-morbidities, no history of use of acid suppressant agents (Khanna et al., 2012). Contrary to what has been reported, Lessa et al found that children are now vulnerable to CDI with more complications and recurrences even when they lack comorbidities and no history of exposure in healthcare settings or antibiotics use (Lessa et al., 2015). Environmental factors as Anderson et al pinpoint may be a risk factor. From their study, it is plausible that proximity to livestock farms may lead to zoonotic transmission of CACDI (Anderson et al., 2017) while Espelage et al observed from their study that isolates in fecal matter from small companion animals like cats and dogs pose a potential zoonotic risk (Rabold et al., 2018). Several studies report that the irrational use of gastric acid suppressants pose a potential risk factor for acquisition of CDI (Tariq, Singh, Gupta, Pardi, & Khanna, 2017), particularly the frequent use of proton pump inhibitors and non-steroidal anti-inflammatory drugs (Dial S, Delaney JAC, Barkun AN, 2005). Nasogastric feeding is potentially a risk factor too as Keefe et al discuss in their study (O'Keefe, 2010)

The possibility of recurrence in CDI is at least in 20-30% of the cases (Tijerina-Rodríguez, Villarreal-Treviño, Morfín-Otero, Camacho-Ortíz, & Garza-González, 2019). This has been associated with a possible elevated production of virulence factors principally toxins A (*TcdA*) and B (*TcdB*) and biofilm formation in an already colonized gut (Dapa & Unnikrishnan, 2013). Virulence factors accord the bacteria antimicrobial resistance whilst preserving the sporulating phase for survival (Janoir, 2015; Crobach et al., 2018; Peng et al., 2017).

Globally, studies have documented different prevalence of community acquired *Clostridium difficile* infection. In America, as of 2016 CACDI prevalence was at 51% (Younas et al., 2020), a 70% increase in incidence was reported in Europe (Davies et al., 2014) with a 13.5% prevalence in 2010 (Jen et al., 2012), 13.6% prevalence in Singapore (Tan et al., 2014), 26% as of 2012 in Australia (Slimings et al., 2014), 0.62% in Kuwait (Jamal, Pauline, & Rotimi, 2015).

Little data is available describing CACDI occurrence and characterization in Sub-Saharan Africa and Kenya in particular. It could be that either the disease burden is low or there is inadequate public health awareness and surveillance or both factors are at play.

Therefore, this study sought to isolate, and identify the associated clinical and microbiological characteristics, the toxins profiles and predisposing factors of *Clostridium difficile*. The study population encompassed adults and children one year and above attending the outpatient departments of the Mbagathi County Hospital and the Mama Lucy Kibaki Hospital within the county of Nairobi over a period of three months. Fecal samples were cultured anaerobically to isolate *C.difficile* and subsequent molecular characterization of the culture positive isolates done.

1.2 Problem Statement

Clostridium difficile infection has gained public health importance owing to its recent increasing prevalence and incidence rates as an infectious disease. More so in the community settings (Guery, Galperine, & Barbut, 2019). *C. difficile* is now identified as a causative agent of infectious diarrhoea in the community away from the hospital settings where in the latter it has surpassed methicillin resistant *Staphylococcus aureus in* the severity index (Miller, Chen, Sexton, & Anderson, 2011; Tang et al., 2016). CACDI equates HACDI in the potential for complicated disease (Clohessy, Merif, & John, 2014), contrasting preexisting epidemiological reports of CDI being principally a nosocomial infection. Recent studies identify CDI's occurrence in populations beyond the defined risk factors like prior admission to a healthcare facility, and antibiotics use, underlying disease and being an elderly. Reported surge in the incidence rates, recurrences, severity score has come in the wake of CDI diagnosis in younger patients mostly females and children, and in whom use of antibiotics or having an underlying medical condition is absent (Sahil Khanna & Gupta, 2014). CDI and its public health importance has also manifested in antimicrobial resistance potentiated by novel epidemic strains exhibiting multidrug resistance especially to recommended therapeutic regimens. This has been a factor in the changing epidemiology and a challenge in the treatment outcomes (Spigaglia, Mastrantonio, & Barbanti, 2018). Globally, the underestimated disease burden has been driven by the glaring differences in the choice of confirmatory diagnosis for CDI. Moreover, the lack of it as a routine diagnostic test. This has been compounded by report bias as most CDI studies are retrospective and hospital-based. Therefore, there is a manifest gap as pertains to scarcity of knowledge on the true incidence, prevalence, associated risk factors and treatment outcomes of CACDI especially in resource-limited settings

4

where diagnostic choices and expertise is a challenge. Patients presenting with watery diarrhoea is a common occurrence in an outpatient hospital setting. This presents with an admixture of fever, headache or abdominal cramping which are typical symptoms of CDI among others. Hence, CDI diagnosis should be a priority routine diagnostic test in an outpatient healthcare setting which is rarely done in this study sites.

1.3 Rationale of study

There is an imperative need for routine clinical diagnosis of *C. difficile* following the numerous scientific reports on the growing incidence and severity, not to mention the need for an active public health surveillance of emerging infectious diseases, given the present consideration of CDI as a primary public health threat more so in the community settings. Diagnosis of *C.difficile* is of utmost importance in epidemiological analysis and the development of control and prevention policies.

The findings from this study serve to fortify existing antimicrobial stewardship programs which aim to preserve antibiotics efficacy and advocacy for cost-effective therapeutic regimens overall limiting the burden on health care costs as recommended from a study by Piacenti et al (Piacenti & Leuthner, 2013). Furthermore, it calls for findings to be integrated in guidelines on surveillance and response programs, infection prevention and control strategies, fortify established guidelines in breaking the environmental cycle in *Clostridium difficile* transmission and foster the development of regional and national health policies, regulations and interventions. Nevertheless, serve to recommend more studies in Kenya and Sub-Saharan Africa where *Clostridium difficile* infection is poorly described and reported.

The findings also challenge the traditional standard infection prevention and control measures implemented to cover HACDI yet CACDI has become a common occurrence as seen by this study by Durovic et al (Durovic & Widmer, 2018). The findings further edify the need for a review of the current treatment options as comparable to the study done by Nelson et al (Nelson, Suda, & Evans, 2017). This study highlights the risk factors of community acquired *C. difficile* infection serving as a clinicians guide on the need for routine diagnosis and hospitalization as an option in management of diarrhoea in an outpatient setting to mitigate development of complications, recurrences, morbidity and mortality; which has been recommended by Khanna et al in their study (S Khanna, Pardi, Aronson, Kammer, & Baddour, 2012). In addition, the findings emphasizes the

public health importance of embracing the one health approach given that domestic animals are a potential risk factor in CACDI (Collins & Riley, 2019).

1.4 Study Questions

I. What is the prevalence of CDI in the community at the study sites?

II. What are the toxins profile of the culture positive isolates?

III. What are the risk factors predisposing to development of CDI in the study population?

1.5 Objectives

1.5.1 Broad Objective

To describe the prevalence, enumerate the risk factors and categorize the toxins associated with community acquired *Clostridium difficile* infection from diarrhoeal samples among outpatients attending the Mama Lucy Kibaki Hospital and the Mbagathi County Hospital.

1.5.2 Specific objectives

IV. To determine the prevalence of community-acquired *Clostridium difficile* infection in patients attending the facilities in the study sites

V. To detect the toxins profile amongst the culture positive *Clostridium difficile* isolates

VI. To describe the risk factors associated with community-acquired *Clostridium difficile* infection

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Virulence

According to Janoir et al the pathogenesis of *Clostridium difficile* associated disease is facilitated by the intrinsic toxins A (TcdA) and B (TcdB) (Janoir, 2016; Kuehne et al., 2010). Siarakas et al noted that toxin B however, may play a bigger role (Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, 2016). There are some genetic variants of *C. difficile* that produce a binary virulent toxin, CDT(Chandrasekaran & Lacy, 2017) essential in adherence (Schwan et al., 2009), the marked severity and mortality too (Gerding, Johnson, Rupnik, & Aktories, 2014). Lyons et al point out that the exotoxins production, secretion is regulated and is strain dependent (Lyon, Hutton, Rood, Cheung, & Lyras, 2016). The proteins, *TcdR, TcdC* and *TcdE* are the regulators (Smits, Lyras, Lacy, Wilcox, & Kuijper, 2016). These toxins destroy the basal structure of the colon, prompting mucosal damage, necrotic inflammation and apoptosis (Aktories, Schwan, & Jank, 2017). *TcdA*, an enterotoxin elicits an inflammatory response by damaging the microvilli tissues, inflammation and necrosis occur, with subsequent acute diarrhoea (Chandrasekaran & Lacy, 2017; Furuya-Kanamori et al., 2015). When TcdB, the cytotoxin, impairs the "tight junctions" of the necrotized epithelium, vascular permeability increases, oedema, and haemorrhage ensues (Carter et al., 2015(Aktories, Schwan, and Jank 2017). Kazawonski et al suggest that that the disrupted signal transduction pathways and the cell cycle dysfunction elicits apoptosis (Kazanowski, Smolarek, Kinnarney, & Grzebieniak, 2014). According to Verhargen et al the binary toxin has been implicated in outbreaks, recurrences and increased incidence rates (Pilate, Verhaegen, Van Ranst, & Saegeman, 2016).

2.2 Asymptomatic carriage

An exposure to *C. difficile* spores lead to asymptomatic *C. difficile* colonization or symptomatic CDI. A growing evidence show asymptomatic *C. difficile* carriers as a source of transmission yet not fully explored. However, the clinical scope of CDI is challenging as the predisposing factors are a common occurrence between the diseased and non-diseased (Furuya-Kanamori et al., 2015). As Hung et al elaborate in their study, asymptomatic carriers of *C. difficile* spores, exhibit similar

environmental and skin contamination to patients diagnosed with CDAD (Hung et al., 2015). However, as Furuya et al investigated and found out, there is inadequate knowledge on the risk factors for asymptomatic *C. difficile* colonization in healthy populations (Furuya-Kanamori et al., 2015). However, when the intrinsic resistance to colonization is overpowered CDI sets in. Stuart et al from their study concluded that asymptomatic colonization is associated with lower risk of CDAD but the risk differs across colonization by either a toxigenic or non-toxigenic strains (Shim, Johnson, Samore, Bliss, & Gerding, 1998). Although asymptomatic colonization promotes host humoral immunity, however, it is a source of acquisition of *Clostridium difficile* infection through horizontal transfer of the toxigenic strains whose frequency of prevalence is dependent on the host, pathogen and environmental factors (Furuya-kanamori et al., 2015). Hung et al point out that predisposing factors for the shift from carriage to CDI, range from a history of a recent healthcare facility admission or an outpatient visit, irrational and prolonged use of antimicrobials and gastric acid suppressants, immunosuppressants, comorbidities like cytomegalovirus infection and Toll-Like Receptor-4 (TLR4) polymorphisms (Hung et al., 2015). They further hypothesized that the serum immunoglobulins expressed in response to *Clostridium difficile* antigens are useful surrogate markers in the shift from asymptomatic carriage, immune response and symptomatic disease (Hung, Lee, & Lin, 2015).

2.3 Zoonotic isolation and transmission

C. difficile infection has been described in pigs, calves, horses, cats and dogs (Bauer & Kuijper, 2015). Bottiger et al from their study suggest that contact with animals is a risk factor for CDI (Søes et al., 2014). A 1.4%- 21.0% rate of colonization with non-human types *C. difficile* isolates has been reported mainly from cats and dogs (Weese, Finley, Reid-Smith, Janecko, & Rousseau, 2010). A study in Kansas City by Hensgens et al established that though *Clostridium difficile* isolates from zoonotic and human populations compare, interspecies transmission is only in the immunocompromised. This was after *Clostridium difficile* "pathogenic and non-pathogenic strains" were obtained from soil, fecal matter, domestic and wild animal's intestinal tracts, reptiles and birds (Hensgens et al., 2012). *C.difficile* strains have also been isolated in beef, chicken and pork raw products (Weese, Avery, Rousseau, & Reid-Smith, 2009). CDI has been shown to asymptomatically colonize animals causing a clinical disease identical to human CDI (Goorhuis et al., 2008).

2.4 Epidemiological changes

Freeman et al established in a study that in the new millennium *Clostridium difficile* infection has been presenting with growing rates of incidence, varying epidemiological reports and a diverse clinical picture (Freeman et al., 2010). The response to treatment and the eventual outcome of *C. difficile* infection have changed too with (Freeman et al., 2010) suggesting that this is due to the wide dissemination of the novel epidemic strains (Khanna, S., & Pardi, 2010) especially in the community settings. In the community settings, reservoirs have been identified to be food sources, water, farm animals and symptomless carriers. The occurrence of CACDI has been underestimated (Khanna et al., 2012), though there is progressive evaluation of the changing epidemiology. Donskey et al suggests that this change could be due to the growing susceptibility of the host to newer predisposing factors, irrational use of antibiotics, the novel *C. difficile* epidemic strains, reservoir in primary asymptomatic carriers and interspecies transmission between humans and animals in the community (Owens, Donskey, Gaynes, Loo, & Muto, 2008; Freeman et al., 2010). Mcdonald et al argues that in developing countries inadequate awareness, diagnostics and non-conventional routine surveillance protocols hinder reports of Community-acquired *Clostridium difficile* infection (L. Clifford McDonald,Bruno Coignard, Erik Dubberke, Xiaoyan Song,Teresa Horan,Preeta K. Kutty, 2007). Available data in Africa on CDI is from hospital based studies magnifying the underestimated burden of CACDI. Seugendo et al (Seugendo et al., 2015) in a research study in Tanzania reported an 8.6% prevalence rate comparable to a study report in Zimbabwe that reported the same prevalence for hospital-acquired CDI. Oyaro et al in their study in Kenya reported a 93.3% prevalence of *Clostridium difficile* infection in two hospital settings (Plants-paris et al., 2019). Kumar et al from their study however reported *Clostridium difficile* infection prevalence in India as an overall 37% but with 33% being community-acquired (Kumar & Uma, 2015). Fellmeth et al in their study (Fellmeth, Yarlagadda, & Iyer, 2010) reported 1.29/10,000 prevalence of CACDI.

2.5 Risk factors

Fellmeth et al propose that the risk factors have shifted from the established to the unusual which can be attributed to the likelihood of zoonotic reservoirs, infants and asymptomatic carriage especially in the community (Fellmeth et al., 2010). Ofori et al suggest that some recent identifiable risk factors for CACDI are largely; younger individuals, females, immunosuppression, unregulated

and prolonged antibiotics exposure, use of gastric acid suppressants and non-steroidal anti-inflammatory drugs, undiagnosed asymptomatic carriage, contaminated food and or water and living within the vicinity to farms . Abdominal surgery, chemotherapy, comorbidities and nasogastric tube feeding cannot be ruled out as predisposing factors to CACDI (Phillips & Hammond, 2017). In the past advancing age (<65 years), hospitalization (within ≤ 4 weeks of diarrhoea onset), regular or concomitant use of broad-spectrum antibiotics, immunosuppression, a history of *Clostridium difficile* infection and comorbidity were the main predisposing factors (Ofori et al., 2018), household transmission between members with CDI or children <2 are risk factors too mainly due to asymptomatic colonization. (Norén et al., 2004; Turner, Smith, & Lewis, 2019)

2.6 Categories of Clostridium difficile associated disease

Gupta, Ofori et al define community-acquired *Clostridium difficile* infection as one whose onset of symptoms occurs in a community setting or within 2 days of hospitalization having not been admitted to a healthcare facility within the last 3 months before the onset of symptoms. Hospital-onset infection is when symptoms settle after hospitalization for ≥ 2 days and or when a month has not elapsed after a hospital discharge or development of symptoms within the community between 4 and 12 weeks after a healthcare facility discharge (Khanna & Gupta, 2014; Ofori et al., 2018)

2.7 Global burden

According to Kotila et al, the case fatality rate of CDI is stipulated as 14% within a month of active disease diagnosis (Kotila, Mentula, Ollgren, Virolainen-Julkunen, & Lyytikäinen, 2016).

Though community-acquired *Clostridium difficile* infection adds to the disease burden of CDI, Miyajima et al claim that it is under diagnosed and under reported (Miyajima et al., 2011). Roldan et al estimate that CDI within Asia and the Middle East could be roughly 10.5% - 19.5% in rates of prevalence. A study in Zimbabwe yielded a prevalence of 8.6%; 9.2% in South Africa where 32% of the diagnosed cases were purported as CACDI; in Nigeria diagnosed cases in the inpatients and outpatients was reported as ≤43% and ≤14% respectively (Roldan, Cui, & Pollock, 2018). In England, a 67% prevalence of CACDI cases in females has reported. In America, population-based studies as reported by Aronson et al approximated prevalence of 33-41% CACDI cases (Ofori et al., 2018). It has been estimated that roughly 20 cases in 100,00o persons-year are CACDI (Chitnis

et al., 2013). Rupnik and Wilcox et al reported that in the United States there are ~500,000 diagnoses of Clostridium difficile among hospitalized patients, with an estimated 15,000 to 20,000 deaths each year whilst the incidence is 14.8 diagnoses in 100,000 people in Germany (Rupnik, Wilcox, & Gerding, 2009)

2.8 Laboratory diagnosis

Patients presenting with diarrhoea should be routinely tested for CDI as a differential diagnosis. Anaerobic culture and isolation on selective agar medium like the cycloserine cefoxitin fructose agar is a gold standard in diagnosis before molecular characterization. Alcohol shocking the stool before media inoculation preserves the spores, killing other enteropathogens and makes isolation easier. Chromogenic agars employed in isolation help in colonies identity under an ultraviolet light (Guery, Galperine, & Barbut, 2019). Light microscopy is commonly used for the morphological identification of *Clostridium difficile*. Nucleic acid amplification tests (NAAT) for *C. difficile* toxin genes are specific and sensitive. According to Yoldas and Crobach et al sequencing and stepwise algorithmic testing of *C. difficile* positive culture is an ideal approach; a screening step for sensitivity followed by specifically detecting the toxins. Like the nucleic acid amplification assay followed by an enzyme immunoassay test or cytotoxicity assay is most reliable and differentiates toxins and non-toxins producing strains (Yoldaş, Altındiş, Cufalı, Aşık, & Keşli, 2016; Guery et al., 2019). The differentiation between toxins and non-toxins producing strains, however maybe a diagnostic challenge as carriage and active infection may lack divisive clarity. Various diagnostic strategies are heterogeneous in reporting CDI incidence rates and genetic variants, reflecting the different phases of CDI spread (Martin, Monaghan, & Wilcox, 2016).

2.9 Treatment

It is imperative that treatment is stratified depending on the severity of the disease. This can either be complicated or severe or the mild or moderate forms. Metronidazole 400mg orally thrice daily for 10 days is recommended for mild to moderate CDI. Vancomycin 125mg six hourly for ten days is recommended for severe disease. Failure to respond to metronidazole within a week the treatment should be alternated with vancomycin for the moderate CDI. Severe and complicated CDI is managed by oral vancomycin 125mg six hourly in combination with intravenous metronidazole 500mg eight hourly (Surawicz et al., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

The study was prospective, cross- sectional conducted during August 2019 to November 2019 in two public health facilities within Nairobi, Kenya.

3.2 Study site

The study was conducted at The Mbagathi County Hospital and The Mama Lucy Kibaki Hospital. The Mbagathi County Hospital is a government public health facility within the Kibra constituency within the Dagoretti Sub-county within the County Government of Nairobi, to the Southwest. The entire constituency has an area of 12.1km2. Kibra is the largest slum in Nairobi and East Africa as well. It is also the largest urban slum in Africa (Mutisya & Yarime, 2011). The population is over 1,000,000 million people as per the 2019 Kenya census report). The hospital serves as a referral health facility within the county of Nairobi for both the slum dwellers and populations from the neighbouring rural towns. The hospital is a Centre of excellence for infectious diseases serving over 3 million people annually both in the outpatient and inpatient departments.

The Mama Lucy Kibaki Hospital is a government referral facility within the Embakasi West constituency and serves more than 4 million people annually. The constituency sits on 208km^2. The constituency has both informal settlements and modern housing. It serves to decongest the referral services needed from other level 5 hospitals within the country. It has both inpatient and outpatient health services serving the urban and rural populations within its vicinity.

3.3 Study Population

The study was non-descriptive and did not set out to compare the characteristics of patients attending any of the two study health facilities and had no target number of patients for each study site. Therefore, upon getting an eligible patient, and getting informed and signed for voluntary participation, the patient was enrolled, questionnaire filled and the diarrhoeal sample collected.

3.3.1 Inclusion criteria

- Individuals with diarrhea aged ≥1 year and above
- Without a history of health care facility admission within 12 weeks prior to the date of the study commencing
- At least three episodes of unformed or watery stool in a 24-hour period

3.3.2 Exclusion criteria

- Any patient unwilling to consent
- Healthcare workers
- Recent abdominal surgery
- Any patient with a history of recurring abdominal pain that has lasted more than 3 months from the day of presentation at the health facility

3.4 Sample size

Although the information on community-acquired *Clostridium difficile* infection is lacking in Kenya, one study in India by (Kumar & Uma, 2015) reported a 33% prevalence in a study population of 145 patients.

Using these proportions as the basis of the calculation for an appropriate sample size using the formula given below;

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

So that:

n is the sample size

Z gives the level of statistical difference as 1.96

P is the prevalence percentage of patients with CDI

d is the estimated error, taken as 0.05

Substituting this in the formula gives a sample size of **340** as shown below:

$$n = \frac{1.96^2 \times 0.33(1-0.33)}{0.05 \times 0.05}$$

$$= 340$$

3.5 Sampling Technique

Consecutive sampling was applied. The study participants were outpatients in the study facilities presenting with diarrhoea to the laboratory for screening. Upon agreeing and signing an informed consent they were enrolled in the study as participants until the desired sample size was achieved.

3.6 Variables

Independent variable measured included age, gender, level of education, occupation, residence and marital status.

Dependent variables included the course of diarrhoea, history of use of antibiotics, concomitant medications, comorbidities, living with a pet, living with an infant, nasogastric tube feeding and history of hospital admission.

3.7 Data Collection Procedures

Data was collected from August to November 2019. We used a structured questionnaire to collect information on the patient's socio-demographic data, the course of diarrhoea, the history of use of antibiotics, the use of concomitant medications, comorbidities, ownership of a pet, living with an infant, history of hospital admission and nasogastric tube feeding. Diarrhoea was considered as any sample that was loose, watery or unformed. The fresh diarrhoeal samples were collected in a clean, dry container and tightly capped and stored at 2-8°c in the fridge before transport in a labeled cool box to the University of Nairobi, School of medicine, department of microbiology , laboratory where the isolation and characterization of *Clostridium difficile* was done. Samples were processed immediately after arrival at the laboratory.

3.8 Laboratory Procedures

3.8.1 Alcohol shocking the diarrhoeal samples

Out of the 340 fecal samples collected, only 301 samples were processed. About 500ul of each fecal sample was transferred to 2mls eppendorf tubes and about the same amount of ethanol (95%) was added to the samples and allowed to stand at room temperature for one hour.

3.8.2 Anaerobic Culture and isolation

The stool samples were inoculated on selective *Brazier's* Cycloserine, Cefoxitin Egg Yolk (*CCEY*) agar plates (Lab M) supplemented with 1% lysed horse blood – cycloserine, cefoxitin and 5% egg yolk emulsion after alcohol shock treatment. Incubation was achieved in 48 hours at 37 °C in an anaerobic GasPak jar (Thermo Fisher Scientific) using anaerobic gas generating sachets (Oxoid). *Clostridium difficile* colonies were identified on the basis of their characteristic horse-stable odour, gram positive rods upon gram staining and characteristics morphology of the colonies. From *C. difficile* positive CCEY, the colonies were stocked in skimmed milk and stored at -80C. The skimmed milk stock isolates were then confirmed by culture on CHROMagarTM *C. difficile* (DRG-International.Inc) and incubated for 48 hours at 37°C anaerobically. Typical *C. difficile* isolates were identified on based on colonial morphology on CHROMagarTM and on fluorescence under an ultraviolet light. The isolates were further stocked on anaerobic fastidious broth (Lab M) and stored at -20°C awaiting DNA extraction.

3.8.3 The extraction of DNA

The culture positive *C. difficile* isolates in broth stock were left to stand for 30 minutes at room temperature after removal from -20°C storage. Afterwards, 50mls of the broth stock was transferred into labeled 50mls conical polypropylene centrifuge tubes. Centrifugation was done at 6000rpm for 5 minutes. The supernatant was discarded and the solid pellet used for DNA extraction according to manufactures instruction using the **Meridian Bioscience ISOLATE II Genomic DNA Kit Code B1052067 (BIOLINE).** The solid pellets in the eppendorf tubes were re-suspended in 200µl of lysis buffer. Afterwards, 25µl of Proteinase K solution and 200µl of lysis buffer were added to the samples. The samples were then incubated at 70°C for 10 minutes and later subjected to vortex for 1 minute and 210µl of ethanol (96%) added. Vigorous vortex of the samples followed for 2 minutes. ISOLATE II Genomic DNA spin column was placed in 2ml

collection tubes. The samples were loaded to the column and centrifuged for 1 minute at 11,000rpm. The flow- through was discarded and the collection tubes reused. Subsequent washing of the silica membrane followed in a two stepwise approach. 500μl of wash buffer was added and centrifuged 1 minute at 11,000rpm. The flow- through was discarded and the collection tubes reused. 600μl of the wash buffer was added, centrifuged 1 minute at 11,000*g and the flow-through discarded and the collection tubes reused. To remove the residual ethanol, a final 1 minute centrifuge at 11,000rpm was done. ISOLATE II Genomic DNA Spin columns were placed in 1.5ml centrifuge tubes. 100μl of preheated elution buffer was added onto the center of the silica membranes, incubated at room temperature for I minute and centrifuged 1 minute at 11,000rpm to a final elution of 50μl of DNA extract.

3.9 Polymerase chain reaction

We performed both singleplex and multiplex convention PCR using the primers published by Lemme et al. (Lemee et al. 2004) to detect the primary toxins and the binary toxins. We first confirmed the C*lostridium difficle* isolates by detecting the *tpi* housekeeping gene on a singleplex assay. Consequently, all *tpi* positive isolates were amplified for *tcd A, tcdB* on a singleplex assay and *cdtA* and *cdtB* on a multimplex assay. For all the assay the reaction volume was 20 μl reaction consisting of 10 μl of dreamTaq, 7 μl of PCR water, 0.5 μl of 10 μM forward and reverse primers and 2 μl of extracted DNA. The cycling conditions included: a cycle of 95°C for 3min, 40 cycles of 95°C for the 30s, 60°C for 30s, and 72°C for 30s.The PCR amplicon products were then confirmed by a gel electrophoresis using a 2% agarose gel at 200 V for 40 minutes and visualized on a UV transilluminator.

3.10 Quality Assurance Protocol

Quality assurance (QA) management plan (Appendix 7) routinely used by the UoN Department of Microbiology ISO certified laboratory and outlines the basic provisions for the personnel, the laboratory facility, procedures for sampling and handling of the samples, maintenance of the equipment used for PCR, quality checks, laboratory reagents and supplies and maintenance of cleanliness in the facility was adopted in this research.

3.11 Ethical committee approval

The proposal was submitted to the Kenyatta National Hospital / UoN Scientific Ethical Committee (KNH-ERC) for scientific and ethical review and was approved (P422/05/2019) prior to inception of the study and the approval letter issued (Appendix 1). A study approval letter was also issued by the Mama Lucy Kibaki Hospital and the Mbagathi County Hospital Research and Training Committees. (Appendix 3 and 4 respectively). The Nairobi City Council Research Department also granted permission for conduct of the study (Appendix 2).

Informed and signed consent was obtained from each study participant (Appendix 5) and for children a guardian had to give informed and signed consent (Appendix 6). The principal investigator and the research assistants took the participants through the context of the study, the risks and benefits of participating, the need for voluntary participation without coercion, and the assurance of privacy and confidentiality of all data collected. All persons found infected with *Clostridium difficile,* the clinicians in the study facilities were alerted and the recommended therapeutic regimens prescribed for the patients. No invasive procedures were carried out during the study.

3.12 Data Management and Analysis

Filled in questionnaires were analysed on a password protected excel sheet then stored away privately and safely in a password coded cabinet accessible only by the principal investigator. The excel sheet data was then exported to Statistical Package for Social Sciences (SPSS) version 20 for statistical analysis. Data was backed up on a password protected external hard drive accessible to the principal investigator only and onto cloud storage in google mail.

Univariate analysis was done using frequencies/ proportions or measures of central tendency for categorical variables. Bivariate analysis was used to test associations using chi-square between predictor and outcome variables. Multivariate analysis using logistic regression to test associations between the key outcome variables with demographics and other independent variables. A 2-sided $P \leq .05$ was considered statistically significant.

3.13 Dissemination of results

The study findings were communicated to the Research and Training committees in the Mama Lucy Kibaki and the Mbagathi County Hospitals. Also, to the Research Department in the Nairobi

City Council. The findings were presented at the UNITID Journal club. A manuscript is being prepared for publication in a peer reviewed open-access journal.

CHAPTER FOUR

4.0 RESULTS

4.1 Social and Demographic characteristics of the study participants

The study had 342 patients with 169/342 (49.4%) males and 173/342 (50.6%) females that were recruited for the collection of demographic and clinical data and diarrhoeal stool samples. The patients were drawn from the outpatient department laboratories in the Mama Lucy and the Mbagathi County hospitals.

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                    ┌─────────────────────────────────────┐
                    │        342 Study Participants        │
                    └─────────────────────────────────────┘

  ┌──────────────────────────┐          ┌──────────────────────────┐
  │  Mama Lucy Kibaki Hospital│          │ The Mbagathi County Hospital│
  │        n= 247            │          │          n=95            │
  └──────────────────────────┘          └──────────────────────────┘

  ┌──────────────────────────┐          ┌──────────────────────────┐
  │   Males= 123 (72.8%)     │          │   Males =46 (27.2%)      │
  │   Females =124 (71.7%)   │          │   Females =49 (28.3%     │
  └──────────────────────────┘          └──────────────────────────┘
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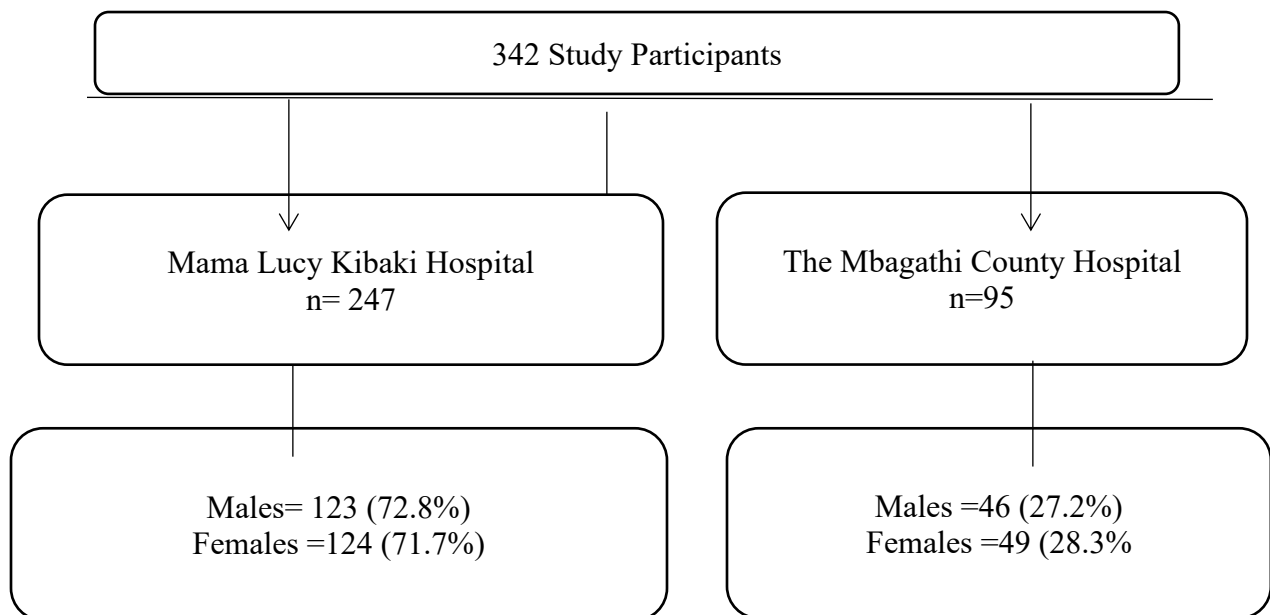
Figure 1: A flow diagram of the frequency of the study participants per study site and the distribution of gender.

Most of the patients were recruited from the Mama Lucy Kibaki Hospital (72%, n=247) while 28%, n=95) were recruited from the Mbagathi County Hospital. One hundred and fifty-nine (46.4%) of the recruited patients live in informal settlements with 103/247 (30.1%) and 56/95 (16.4%) visiting the Mama Lucy Kibaki and the Mbagathi County hospitals respectively. Majority of the study participants resided in the informal settlements 159/342 (46.5%). The age of the participants ranged from 1-62 years with an interquartile (IQR) from 14 to 38 years. The median

age of the recruited participants (n=342) in the study was 27.5 ± 15.7 years. Participants at the Mama Lucy hospital (n=247) and the Mbagathi county hospital (n=95) had an average age of 27.4 ±15.3 years and 26.9 ± 16.8 years respectively. About 136/342 of the participants (39.8%) are married. Although, 124/342 (36.3%) of the study participants have attained a tertiary education, the level of unemployment remains high as 199/342 (58.2%) participants are unemployed. At least 113/342 (33%) participants had a secondary education with 85/342 (24.9%) being salaried while 58/342 (17.0%) are self-employed.

Table 1: Socio- demographic characteristics of the study participants

Characteristics	Description	Study sites n (%)		Total
		Mama Lucy Kibaki	Mbagathi	
Age in years	≤2	9 (19.6%)	4 (9.3%)	13 (28.9%)
	3-18	58 (17.0%)	28 (8.2%)	86 (25.1%)
	19-35	100 (29.2%)	30 (8.8%)	130 (38.0%)
	36-50	61 (17.8%)	24 (7.1%)	85 (24.9%)
	Above 50	19 (5.6%)	9 (2.6%)	28 (8.2%)
Gender	Male	123 (36.0%)	46 (13.5%)	169 (49.4%)
	Female	124 (36.3%)	49 (14.3%)	173 (50.6%)
Marital status	Married	99 (28.9%)	37 (10.8%)	136 (39.8%)
	Single	78 (22.8%)	30 (8.8%)	108 (31.6%)
	Child	65 (19%)	27 (7.9%)	92 (26.9%
	Widowhood	5 (1.7%)	1 (0.3%)	6 (2%)
Level of Education	Primary	56 (16.4%)	24 (7%)	80 (23.4%
	Secondary	78 (22.8%)	35 (10.2%)	113 (33%)
	Tertiary	102 (29.8%)	22 (6.4%)	124 (36.3%)
	Non-school going	11 (3.2%)	14 (4.1%)	25 (7.3%)
Occupation status	Employed	59 (17.3%)	26 (7.6%)	85 (24.9%)
	Unemployed	139 (40.6%)	60 (17.5%)	199 (58.2%)
	Self-employed	49 (14.3%)	9 (2.6%)	58 (17.0%)
Residence	Urban	24 (7.0%)	3 (0.9%)	27 (7.9%)
	Semi-urban	104 (30.4%	31 (9.1%)	135 (39.5%)
	Informal	103 (30.1%	56 (16.4%)	159 (46.5%)
	settlement	16 (4.7%)	5 (1.5%)	21 (6.1%)
	Rural			

4.2 Socio-Demographic and clinical characteristics of patients with and without CACDI

A total of 301 diarrhoeal samples were processed out of which 36/301 (11.96%) were culture positive for *C. difficile* of which 21 (58.3%) and 15 (41.7%) were males and females respectively. The youngest participant being 1 year and the oldest 52 years at which the median age was 24.5 years with an interquartile range from 14.5 to 35 years. Average age was 24.92 ±13.7 years. Among the age groups with CACDI infants (1-2 years) were; 1/301 (0.3%) 3-18 years were 10/301 (3.3%); 19 to 35 years were 18/301 (6.0%), 36-50 years were 6/301 (2.0%), and above 50 years were 1/301 (0.3%). The consistency of the stool samples among the CACDI culture positive patients in the majority was, yellow, unformed and mucoid 7/36 (19.4%). There was no significant association between the variables analyzed for the C. difficile culture positive and the negative cases for variables including age (p=0.80), gender (0.266), the study site (p=0.18), living with an infant in the same household (p=0.76), the use of prescription medicines like antihypertensives, antidiarrhoeals, antispasmodics, and antiretrovirals (p=0.76), clinical symptoms like fever, headache, bloating and acidity, and loss of appetite (p=0.85). However, factors including, duration of diarrhoea (p=0.04), the use of antibiotics within a month before the onset of the diarrhoea (p=0.03), use of gastric acid suppressants (p=0.002), use of chemotherapeutic agents (p=0.01), use of NSAIDs (p=0.02), HIV/AIDS comorbidity (p=0.02), abdominal cramping (p=0.03), gastrointestinal bleeding (p=0.05), organ transplant (p=0.004), and having a small companion or production animal in the household (p=0.03), showed significant association as summarized in table 2 below.

Table2; Socio-Demographic and clinical characteristics of the study participants positive and negative for CACDI

Variables	Description	CACDI Positive n= 36) N (%)	CACDI negative (n=265) N (%)	Total (n=301) N (%)	P-Value
Age group in years	≤2 years	1 (0.3%)	8 (2.7%)	9 (3.0%)	0.80
	3-18	10 (3.3%)	66 (21.9%)	76 (25.2%)	
	19-35	18 (6.0%)	87 (28.9%)	105 (34.9%)	
	36-50	6 (2.0%)	54 (17.9%)	61 (19.9%)	
	Above 50	1 (0.3%)	50 (16.6%)	51 (16.9%)	
Gender	Male	21 (7.0%)	126 (41.9%)	147 (48.8%)	0.26
	Female	15 (5.0%)	139 (46.2%)	154 (51.2%)	
Study site	Mama Lucy	26 (8.6%)	200 (66.4%)	226 (75.0%)	0.98
	Mbagathi	10 (3.3%)	65 (21.6%)	75 (24.9%)	
Duration of diarrhoea	<1 week	28 (9.3%)	175 (58.1%)	203 (67.4%)	0.04
	1-3 weeks	7 (2.3%)	65 (21.6%)	72 (23.9%)	
	>3 weeks	1 (0.3%)	25 (8.3%)	26 (8.6%)	
Farm or Small companion animals kept	Yes	13 (4.3%)	43 (14.3%)	56 (18.6%)	0.03
	No	23 (7.6%)	222 (73.8%)	245 (81.4%)	
Living with an infant	Yes	2 (0.6%)	15 (5.0%)	17 (5.6%)	0.76
	No	34 (11.3%)	250 (83.1%)	284 (94.4%)	
HIV/AIDS comorbidity	Positive	7 (2.3%)	21 (7.0%)	28 (9.3%)	0.02
	Negative	29 (9.6%)	244 (81.1%)	273 (90.1%)	
Antibiotics used before diarrhoea onset	Cephalosporins	0 (0.0%)	5 (1.7%)	5 (1.7%)	0.03
	Fluoroquinolones	10 (3.3%)	20 (6.6%)	30 (10.0%)	
	Penicillins	6 (2.0%)	39 (13.0%)	45 (15.0%)	
	Metronidazole	1 (0.3%)	25 (8.3%)	26 (8.6%)	
	Macrolides	0 (0.0%)	5 (1.7%)	5 (1.7%)	
	None	19 (6.3%)	171 (56.8%)	190 (63.1%)	
Use of acid suppressants	PPIs	8(2.7%)	16 (5.3%)	24 (7.0%)	0.002
	H2RAs	0 (0%)	2 (0.6%)	2 (0.6%)	
	Magnesium tabs	0 (0%)	1 (0.3%)	1 (0.3%)	
	None	28 (9.3%)	246 (81.7%)	274 (91.0%)	
Use of chemotherapeutic drugs	Yes	2 (0.6%)	0 (0.0%)	2 (0.6%)	0.01
	No	34 (10.0%)	265 (88.0%)	299 (99.3%)	
NSAIDs use	Yes	6 (2.0%)	17 (5.6%)	23 (7.6%)	0.02
	No	30 (10.0%)	248 (82.4%)	278 (92.4%)	
Abdominal cramping	Yes	15 (5.0%)	164 (54.5%)	179 (59.5%)	0.03
	No	21 (7.0%)	101 (33.5%)	122 (40.5%)	

Additional symptoms	Fever	3 (1.0%)	53 (17.6%)	56 (18.6%)	
	Headache	3 (1.0%)	49 (16.3%)	52 (17.3%)	0.85
	Bloating	28 (9.3%)	146 (48.5%)	174 (57.8%)	
	Bloody stool/	12 (4.0%)	45 (15.0%)	57 (18.9%)	
	gastrointestinal	24 (8.0%)	220 (73.0%)	244 (81.1%)	0.05
	bleeding				
Other prescribed	Antidiarrheal	2 (0.6%)	21 (7.0%)	23 (7.6%)	
medications	Antiretrovirals	1 (0.3%)	3 (1.0%)	4 (1.3%)	
	Antihypertensives	0 (0%)	2 (0.6%)	2 (0.6%)	0.76
History of organ transplant	Yes	1 (0.3%)	0 (0%)	1 (0.3%)	0.004
	No	35 (11.6%)	265 (88.0%)	300 (99.7%)	
History of bowel surgery	Yes	0 (0%)	1 (0.3%)	1 (0.3%)	0.73
	No	36 (12.0%)	264 (%)	300 (99.7%)	

Abbreviations: NSAID- Non-Steroidal Anti-inflammatory drugs; CACDI -Community-acquired

Clostridium difficile infection

4.3 Toxin profile of C. difficile isolates

Of the 36 *C. difficile* isolates, (35/36, 97.2%) (35/301. 11.6%) were toxigenic

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Toxigenic strain	CACDI N=36	CACDI N=301
tcdA+/tcdB+/CDTa-/b-	18 (50%)	18 (6.0%)
tcdA+/tcdB+/CDTa+/b+	9 (25%)	9 (2.9%)
tcdA-/tcdB+/CDTa-/b-	5 (14%)	5 (1.6%)
tcdA-/tcdB+/CDTa+/b+	3 (8%)	3(1.0%)
tcdA-/tcdB-/CDTa-/b-	1 (3%)	1(0.3%)
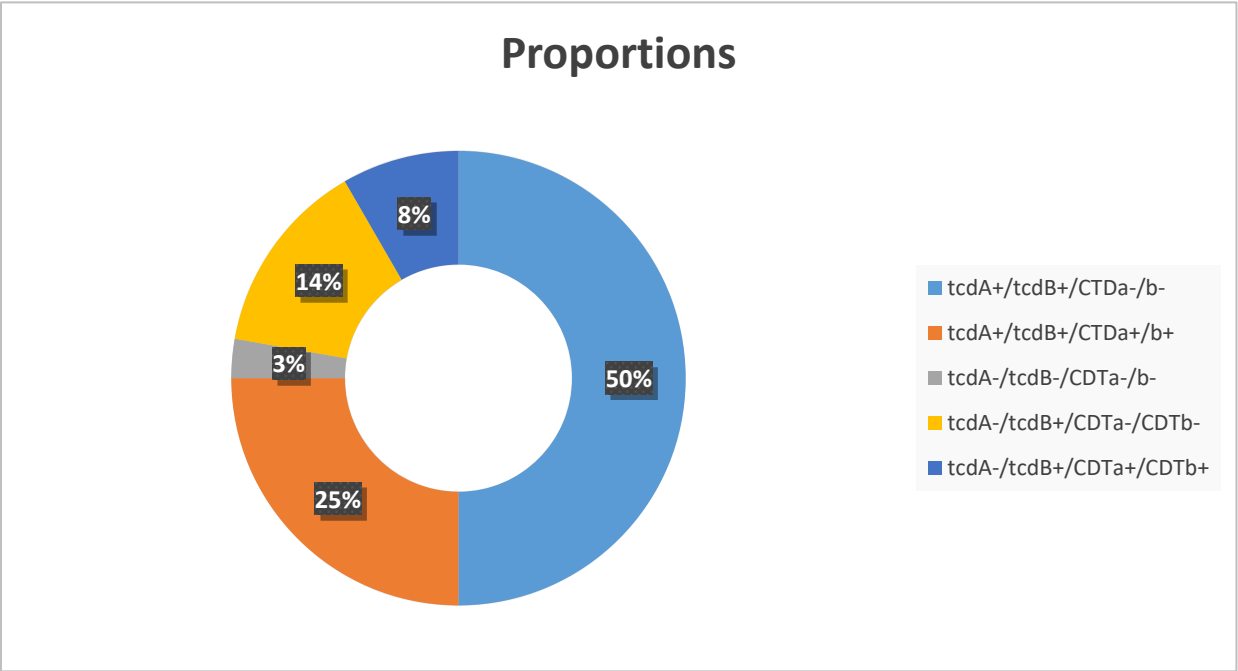
Figure 2: Frequency of the toxins profile

4.4 Determining the risk factors associated with CACDI

This study was conducted to determine the various risk factors that predispose an individual to *Clostridium difficile* infection in the community. It was hypothesized that gender, having chronic diarrhoea accompanied by abdominal cramping and bleeding in the gut, the frequent use of certain broad spectrum antibiotics and acid suppressants drugs, the use of chemotherapeutic drugs, the use of non-steroidal anti-inflammatory drugs, having HIV/AIDS as a comorbidity and the rearing of small companion or production animals will positively predict a CDI infection. To test this hypothesis, binary logistic regression analysis was executed to determine the association between the risk factors and a positive *Clostridium difficile* infection.

On univariate analysis all other variables except use of antibiotics, NSAIDs, chemotherapeutic drugs, having a farm or domestic animal, history of an organ transplant, having abdominal cramping, were significantly associated with acquisition of community acquired *Clostridium difficile* (p≤.05). However, for all other confounders, patients on NSAIDs, with abdominal cramping episodes, use of metronidazole, use of macrolides, use of chemotherapeutic drugs and

organ transplant recipients were more likely to present with CACDI in comparison to those without as summarized in table 3 below.

Table 3: Logistic regression analysis of risk factors associated with CA-CDI

Variables	COR	95% C.I Lower	95% C.I Upper	P-value	AOR	95%C.I lower	95%C.I upper	Chi square	CACDI %
Use Of NSAIDs				0.004				0.02	
Yes	0.295	0.108	0.805	0.004	7.125	1.843	27.55	0.02	6 (1.8%)
No				0.004	Ref	Ref	Ref	0.02	30 (8.8%)
Use of antibiotics				0.075				0.03	
Cephalosporins	0.987	0.153	0.435	0.075	0.001	0.234	0.356	0.03	0 (0.0%)
Fluoroquinolones	0.340	0.062	.0073	0.075	0.170	0.053	0.546	0.03	10 (1.5%)
Penicillins	1.233	0.567	1.790	0.075	0.930	0.310	2.790	0.03	6 (1.8%)
Metronidazole	1.925	0.435	7.623	0.075	1.683	0.292	9.694	0.03	1 (0.3%)
Macrolides	1.463	1.627	1.923	0.075	1.243	1.546	1.876	0.03	0 (0%)
None				0.075	Ref	Ref	Ref	Ref	19 (5.6%)
HIV/AIDS				0.001				0.02	
Yes	3.733	1.58	8.80	0.001	0.153	0.054	0.433	0.02	7 (2.1%)
No	5.789	3.233	6.55	0.001	Ref	Ref	Ref	0.02	29 (6.2%)
Chemotherapeutic drugs use				p>0.05				0.01	
Yes	1.22	0.658	2.268	p>0.05	0.944	0.473	1.022	0.01	2 (0.6%)
No	1.07	0.368	3.107	p>0.05	Ref	Ref	Ref	0.01	34 (10.0%)
Organ transplant history				0.04				0.004	
Yes	9.714	7.098	13.26	0.04	1.22	0.658	2.268	0.004	1 (0.3%)
No	8.769	2.567	28.00	0.04	Ref	Ref	Ref	0.004	35 (10.3%)
Bloody stool				0.003				0.05	
Yes	0.333	0.035	3.195	0.003	0.317	0.033	0.003	0.05	12 (3.5%)
No	1.255	0. 235	0.635	0.003	Ref	Ref	Ref	0.05	24 (7.1%)
Abdominal cramping episode				0.002				0.03	
Yes	3.792	1.1353.	2.346	0.002	5.002	1.915	13.068	0.03	15 (4.4%)
No	7.345	3.239	4.786	0.002	Ref	Ref	Ref	0.03	21 (6.2%)
Farm or domestic animal				0.025				0.03	
Yes	2.171	1.042	4.525	0.025	0.392	0.162	0.949	0.03	
No	2.899	1.349	6.188	0.025	Ref	Ref	Ref	0.03	13 (3.8%) 23 (6.7%)

Duration of diarhoea				0.03				0.04	28 (8.2%)
1 week	0.007	0.125	0.139	0.03	0.081	0.008	0.864	0.04	7 (12.1%)
1.3 weeks	0.008	0.102	0.114	0.03	0.230	0.020	2.609	0.04	1 (0.3%)
>3 weeks	0.169	0.054	0.063	0.03	Ref	Ref	Ref	0.04	
Use of acid suppressants				0.005				0.002	
PPIs	1.234	0.765	0.075	0.005	0.177	0.068	0.460	0.002	8 (2.3%)
H2RAs	5.768	1.567	1.234	0.005	1.000	0.999	0.990	0.002	0 (0%)
Magnesium trisilicate	4.472	1.456	1.198	0.005	1.000	0.989	0.789	0.002	0 (0%)
None	2.355	1.345	1.456	0.005	Ref	Ref	Ref	0.002	28 (8.2%)

Adjusted variables included; the duration of diarrhoea, the use of gastric acid suppressants, the use of antibiotics, the use of NSAIDs, the use of chemotherapeutic drugs, having a farm or small companion animal (pet), the history of an organ transplant, presenting with abdominal cramping and presenting with bloody stool.

CHAPTER FIVE

5.0 DISCUSSION

This prospective cross-sectional study set out to Isolate and characterize toxigenic *Clostridium difficile* strains within community settings in order to establish the disease burden and its specific associated predisposing factors. CDI presents with an acute onset of watery diarrhoea with an admixture of fever, abdominal cramping and prolonged periods of diarrhoea and or muco-hemorrhagic consistency. In this current study, abdominal cramping as a prominent symptom was significant (p=0.01), gastrointestinal bleeding was significant too in diagnosis (p=0.02), as well as the duration of diarrhoea (p=0.03). The prevalence of CACDI in this study was 11.96%. This finding complements other prevalence studies carried out globally like in Nigeria, 14% prevalence (Onwueme et al., 2011), 8.6% in Zimbabwe (Simango & Uladi, 2014) and 12.1% in Korea (Lee et al., 2018). These prevalence fall within the stipulated prevalence rates of 5-45% of CDI in Sub-Saharan Africa. Some studies, however have reported higher prevalence rates of CACDI than this study especially in the developed countries. Of all CDI cases reported in the US, Canada and Europe, 20-27% are CACDI (Wilcox, Mooney, Bendall, Settle, & Fawley, 2008). In Australia, higher rates of prevalence have been reported at 29% (Clohessy, Merif, & Post, 2014), 41% in America (Khanna et al., 2012), 76.5% in Japan (Mori & Aoki, 2015)74.6% in the UK (Taori, Wroe, Hardie, Gibb, & Poxton, 2014), whilst other findings beyond the Sub-Saharan Africa compare with our findings; 11.5% in Korea (Kwon et al., 2017), and others are on a lower scale like this 5.1% prevalence rate reported in China (Ho et al., 2017). These varying rates in prevalence establish that CDI is beyond the nosocomial confines in transmission and carriage. Novel transmission pathways remain largely unexplored especially in the community. This supports the narrative that the epidemiological landscape of CDI is evolving and so is the public health importance which has been intensifying in the last four decades (Poxton, 2013). The varying rates of prevalence suggest that the choice of the diagnostic tool and the study settings may have a role. Another challenge being the misclassification of CACDI as HACDI, inadequate surveillance as largely in most geographical zones the populations at risk is not well defined (Turner, Smith, & Lewis, 2019).

The current study findings were that the probability of having Community- acquired *Clostridium difficile* increased among younger persons. It was realized that in this present study had the oldest patient at 52 years and the youngest at one year with a median age of 24.5 years which agrees with

most findings that CACDI is prevalent among the younger persons as opposed to advancing age. This finding is congruent with (Chitnis et al., 2013) findings that in the last decade CDI has been frequently reported in the young healthy population without conventional factors like comorbidities in the community. In this study, among the CACDI positive persons, none had the predisposing underlying medical conditions like bowel disease or a malignancy. However, 5.6% had HIV/AIDs which has been thought of a risk factor in CDI as immunosuppression fosters bacterial infections with subsequent frequent hospital admissions and antibiotics use increasing the chances of CDI. As (Collini, Kuijper, & Dockrell, 2013) alludes that CDI has a higher incidence in HIV seropositive persons. In this present study, all participants 18 years and below were categorized as children where 2.8% were infants. Usually, either colonization by non-pathogenic strains or the less virulent pathogenic strains, the innate immunity factors in their gut and passive immunity in breast milk makes them a low risk CDI population. However, acute onset but prolonged diarrhoea has been CDI associated, exemplifying the findings that infants and newborns are also at a risk of active infection although the significance in pathogenesis is still unclear. From the findings of a study by (Borali et al., 2015) we find that the disease burden in paediatric CDI is community acquired. Therefore, cases of prolonged bloody and or mucoid diarrhoea in infants, CDI should be routinely diagnosed. From this present study, the oldest patient with CACDI was 52 years and the youngest was one, hence, would be justified to suggest that CACDI is in all age groups and gender is not a denominator. Our study findings noted a 58.3% dominance of males and this is consistent with a 52% prevalence in a study in Iran (Goudarzi et al., 2013) which highlights the male gender as a primary risk factor in CDI probably due to inadequate hand washing, which favours fecal-oral transmission or contact with spores contaminated surfaces, thus, opposing the argument by (Khanna, Pardi, Aronson, Kammer, & Baddour, 2012) that females seek medical attention more than men, therefore risking healthcare exposure and the transmission from infants with frequent contact as primary caregivers (Ofori et al., 2018). However, differences in age and gender showed no statistical significance in this study. In most studies, however, a hypothesis has not been offered for the discrepancy in CACDI across the gender divide.

The use of gastric acid suppressants as a risk factor in CACDI was evaluated in this present study with a 2.3% prevalence (p=0.03) reported with the use of proton pump inhibitors. These findings

complement the study by (Dial S, Delaney JAC, Barkun AN, 2005) that reported a 2.9% PPIs use and CACDI occurrence while a report by (Pant, Madonia, & Minocha, 2009) hypothesize that their use leads to loss of host defense mechanisms, consequently proliferation of the spores and infectivity follows (Imhann et al., 2016; Seto, Jeraldo, Orenstein, Chia, & DiBaise, 2014). The association of PPIs use and CDI can be a greater proportion than in our findings increasing the likelihood of PPIs as a primary factor in CACDI as seen in (Bloomfield & Riley, 2016) findings of 18%. However, the role of PPIs in CACDI has been obviated in as elaborated in a case control study on community- acquired CDI by (Naggie et al., 2011). This current study showed significant association between PPIs use and CACDI.

The possibility of zoonotic transmission as a risk factor was evaluated in this present study. CACDI. Among the positive cases, a 3.5 % (p=0.03) association was reported. These findings are congruent with the findings from a study by (Rabold et al., 2018) which reported a 3.0% and 2.9% risk of association between small companion animals and their owners acquiring CACDI. Animals show asymptomatic carriage of *C. difficile* especially in younger years, proliferation in maturity especially where antimicrobials are administered prophylactically and as growth promoters increasing their susceptibility to CDI. Although, there is inadequate direct evidence, circumstantially there is a potential of zoonotic transmission (Hensgens et al., 2012). Exposure to specific antibiotics like clindamycin, fluoroquinolones and cephalosporins has been reported to be a major risk factor in CACDI acquisition. In this current study no significant association was reported between the use of antibiotics like cephalosporins, fluoroquinolones, penicillins and metronidazole (p=0.075) and CACDI which compares with (Bauer et al., 2008) findings. From other findings a 50% of CACDI occurrence is in similar setting has been reported where in 12 weeks before onset of symptoms there was no antibiotics exposure (Bloomfield & Riley, 2016). Antibiotics exposure maybe an important factor in CACDI especially with clindamycin, fluoroquinolones and cephalosporins (Deshpande et al., 2013), however they may not be essential as other undetermined factors may play a role. Proportions of 32-36% and 43-65% in populations affected with CACDI have been reported where exposure to antibiotics was non-essential in the development of the infection (Chitnis et al., 2013).

In this current study, the use of non-steroidal anti-inflammatory drugs (NSAIDs) (p=0.005) was found to be significant in CACDI with a 10.6% frequency among the CDI patients. Our findings

are congruent with this study (Permpalung, Upala, Sanguankeo, & Sornprom, 2016) that reports of significant increased odds of CACDI in patients exposed to NSAIDs. This study found that NSAIDs exposure exhibited 7.1 times more likely in a person to develop CACDI. NSAIDs have been shown to alter the gut microbiome allowing proliferation of *Clostridium difficile* and associated pathogenesis.

Characterization of toxigenic and non-toxigenic strains depends on the virulence genes expressed. Main virulence factors of *Clostridium difficile* being the expression of toxin genes of the enterotoxin, toxin A (*tcdA)*, the cytotoxin, toxin B (*tcdB*) and the binary toxin *CDTa* and *CDTb*. The findings from this current study reported an 11.6% of the toxigenic strains.

All the 36 CACDI isolates had the *tpi* gene (100%). The toxins profile observed were; *tcdA+/tcdB+/CDTa-/b-* 18 (50%), *tcdA+/tcdB+/CDTa+/b+* 9 (25%), *tcdA-/tcdB-/CDTa-/b-* 1 (3.0%) and *tcdA-/tcdB+/CDTa-/b-* 5 (14%), *tcdA-/tcdB+/CDTa+/b+* 3 (8%). These strains have significance in the pathogenesis of CACDI.

This study had some limitations some of which were limiting the study sites to two public health facilities and not including a private health facility just to assess the significance between the middle and low income class citizens and CACDI prevalence. Bias might have been introduced in gathering of clinical data through the questionnaire where language barrier, illiteracy or other factors may have hindered the objectivity of the study through subjective bias.

6.0 CONCLUSION

In Kenya, this is the first study on the prevalence of *Clostridium difficile* infection within the community settings. The findings are an indication that CDI epidemiology and the predisposing factors vary from what has been reported globally. These findings highlight the need for awareness, development of stepwise diagnostic algorithms whilst embracing the laid down policies and guidelines on routine diagnosis and management, advocate for rational use of antibiotics through antimicrobial stewardship programs whilst maintaining an active public health surveillance entity to monitor the changing epidemiological scope primarily to establish its impact in the public health sphere. A high clinical suspicion index is required among the healthcare professionals to improve on the diagnosis, define the disease burden and its impact and implement measures to contain CDI.

7. 0 RECOMMENDATION

Asymptomatic carriage of *Clostridium difficile* may be well documented though the impact of toxigenic and non-toxigenic strains and pathological relevance is unclear warranting more research on a larger scale to establish incidence rates, define the significant risk factors, characterize emerging strains, and illustrate the antimicrobial susceptibility profile of *Clostridium difficile* to curb the growing menace of antibiotic resistance.

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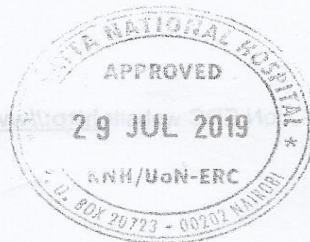
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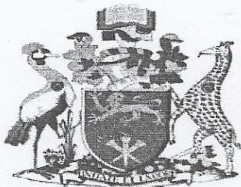
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29ᵗʰ   July, 2019

Ruth Wandia Maina
Reg. No.W64/8348/2017
Institute of Tropical and Infectious Diseases (UNITID)
College of Health Sciences
University of Nairobi

Dear Ruth

Research Proposal: Clinical and Microbiological Characteristics of Community –Acquired
***Clostridium Difficile* Infection among Patients in selected health facilities within Nairobi County**
(P422/05/2019)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 29ᵗʰ July 2019 – 28ᵗʰ July   2020.
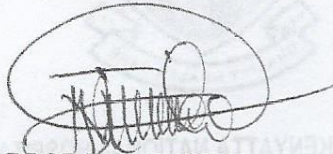
This approval is subject to compliance with the following requirements:

 a.   Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
 b.   All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
 c.   Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
 d.   Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
 e.   Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
 f.   Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
 g.   Submission of an *executive summary* report within 90 days upon completion of the study.
    This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

31

For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Yours sincerely,

PROF.M.L. CHINDIA
SECRETARY, KNH-UoN ERC

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        The Director, CS, KNH
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24th September 2019

Dr. Ruth Wandia Maina
University of Nairobi

RE: RESEARCH AUTHORIZATION

This is in reference to your application for authority to carry out a research on
"Clinical and Microbiological Characteristics of Community – Acquired Clostridium Difficile Infection among Patients in selected Health Facilities Within Nairobi County"

I am pleased to inform you that your request to undertake the research in the hospital has been granted.

On completion of the research you are expected to submit one hard copy and one soft copy of the research report / thesis to this office.

Dr. D. Kimutai
Chairman – Research Committee
Mbagathi Hospital

NAIROBI CITY COUNTY

COUNTY HEALTH SERVICE

Ruth Wandia

Reg. No W64/8348/2017

Nairobi University

RE: RESEARCH AUTHORIZATION

This is to inform you that the Nairobi City County Operational Technical Working group reviewed the documents on the study titled, " **Clinical and Microbiogical Characteristics of Community – Acquired Clostridium Infection among Patients in selected Health within Nairobi County"**. I am pleased to inform you that you have been authorized to undertake the study in Nairobi County.

The researcher will be required to adhere to the ethical code of conduct for health research in accordance to the Science Technology and Innovation Act, 2013 and the approval procedure and protocol for research for Nairobi County

On completion of the study, you will submit one hard copy and one copy in PDF of the research findings to our operational research technical working group.

Raphael Muli

FOR COUNTY DIRECTOR OF MEDICAL SERVICES

CC: **All Sub County SCMOH's**

ALL Medical Superintendents

REPUBLIC OF KENYA
MINISTRY OF HEALTH
NAIROBI CITY COUNTY

MAMA LUCY KIBAKI HOSPITAL-EMBAKASI
P.O. Box 1278-00515
NAIROBI

OUR REF: MLKH/ADM/RES/1/4/(   )

DATE: 23rd August, 2019

Dr. Ruth Wandia Maina
P. O. BOX 69391-00400
NAIROBI

RE: TEMPORARY PERMISSION TO COLLECT DATA

TITLE: "CLINICAL AND MICROBIOLOGICAL CHARACTERISTICS OF COMMUNITY-ACQUIRED CLOSTRIDIUM DIFFICILE INFECTION AMONG PATIENTS IN SELECTED HEALTH FACILITIES WITHIN NAIROBI COUNTY"

Refer to your application to collect data on the above research in this institution.

This is to inform you that the hospital has given you temporary permission to allow you collect data which expires after the next Research Committee Meeting.

DR. MUSA MOHAMMED
MEDICAL SUPERINTENDENT

APPENDIX 6a - Study Questionnaire (English version)

Health facility --------------------------------

Date ---

Patient identification NO. ---------------------

PART A

DEMOGRAPHICS (Please tick the most correct)

1). Gender   O Male   O Female

2). Area of residence

O Urban   O Semi-urban   O Informal settlement   O Rural

3). Year of birth ---

4). Marital status

O Married   O Unmarried   O Single   O Child   O Divorced O Deceased O Separated

5). Level of education

O Primary   O Secondary   O College and above

6). Employment

O Employed   O Unemployed   O Self-employed

If employed what type of occupation----------------------------

PART B

THE COURSE OF DIARRHOEA

1). Have you experienced diarrhoea?

O Yes   O No   O Unknown

2).For how long have you had diarrhoea?

O <1 week   O 1 to 3 weeks   O >3 weeks   O Unknown

3).Did you have stomach cramps?

O Yes   O No   O Unknown

4).Was the stool bloody?

O Yes   O No   O Unknown

5).What other symptoms are you experiencing?

     o   Fever

o Vomiting

o Gas

o Bloating

o Others

6).Onset date of the symptoms ………………………………..

PART C

HISTORY OF ANTIBIOTICS USE

1). Are you taking antibiotics currently?

O Yes   O No   O Unknown

2). If the answer is "yes"

Name …………………………………   Date started ………………………………

3). Number of antibiotics in 30 days before the onset of the diarrhoeal episodes

1 O   2 O   3 O   4 O   5 O   6 O   >6 O

4).In the last month

Cephalosporin   O

Fluoroquinolones   O

Amoxicillin/ Ampicillin   O

Metronidazole   O

Vancomycin   O

Other   O

PART D

CONCOMITANT MEDICATIONS

1). Have you taken acid suppressants in the last one month?

O Yes   O No

If "yes" please specify ---

2). Are you receiving any chemotherapeutic drugs?

O Yes   O No

If "yes" please specify ……………………….

3). Are you on any painkillers?

O Yes   O No

If "yes" please specify…………………………

4). Are you on any other prescription medication?

O Yes   O No

If "yes" please specify …………………………………………

5). Have you taken any medication to stop the diarrhoea

O Yes   O No

If "Yes" please specify ………………………………………..

6). Are you hypertensive and on medication?

O Yes   O No

7). Are you diabetic and on medication?

O Yes   O No

8). Have you been diagnosed with tuberculosis and on medication?

O Yes   O No

PART E

COMORBIDITIES

1). Do you suffer from or have suffered from a medically diagnosed bowel disease within the last 3 months?

O Yes   O No

If "yes" please specify ………………………………..

2). Have you been diagnosed with any malignancy

O Yes   O No

If "yes" please specify …………………………………………

3). Do you have HIV/AIDS?

O Positive   O Negative   O Unknown

4). Have you had an organ transplant?

O Yes   O   No

5). Have you had bowel surgery?

O Yes   O No

PART F

OTHER FACTORS

1). Do you rear any farm animals or live with a pet?

O Yes   O No

If "Yes" please specify ……………………….

2). Have you had an overnight stay in a healthcare facility within the last year?

O Yes   O No

3). Have you had an episode of nasogastric tube feeding?

O Yes   O No

4). Do you have an infant in your household?

O Yes   O NO

APPENDIX 6b-Study Questionnaire (Kiswahili version)

Hospitali husika …………………………….

Tarehe …………………………………………

Nambari ya mhusika ………………………..

SEHEMU YA KWANZA

DEMOGRAFIA (TAFADHALI CHAGUA JIBU LILO SAHIHI)

1).Jinsia

O Kiume   O Kike   O Sitambui

2).Makazi

O Mjini   O Nusu- mjini   O Kijijini   O Kibandani

3).Siku na mwaka wa kuzaliwa ……………………

4).Hali ya ndoa

O Nimeolewa   O Sijaolewa   O Mtoto   O Talaka   O Nimefiwa

5).Kiwango cha masomo

O Shule ya msingi   O Shule ya sekondari   O Elimu ya juu

6).Ajira

O Nimeajiriwa   O Sijajairiwa   O Mwanabiashara

Kama umeajiriwa, unafanya kazi jinsi gani? ………………….

SEHEMU YA PILI

KOZI YA KUHARA

1).Unaendesha?

O Ndio   O La

2).Muda umeendesha?

O Chini ya wiki moja   O Wiki moja mpaka tatu   O Chini ya wiki tatu   O Sijui

3).Tumbo linauma?

O Ndio   O La

4).Umewahi kuona damu kwa choo yako?

O Ndio   O La   O Sijui

5).Uko na dalili zingine za ugonjwa?

O Joto

O Kutapika

O Gesi

O Kuvimba tumbo

O Ingine

6). Tarehe dalili zilianza? ………………………….

SEHEMU YA TATU

MUDA WA KUTUMIA ANTIBIOTIKI

1).Unatumia antibiotiki hivi sasa?

O Ndio   O La

2).Kama ndio?

Jina la dawa …………………   Tarehe ulianza ………………….

3).Nambari ya aina ya antibiotiki siku 30 zimepita kabla uanze kuhara?

O 1   O 2   O 3   O4   O 5   O 6   O>6

4).Mwezi umepita umekunywa

O Cephalosporin

O Fluoroquinolone

O Amoxicillin/ Ampicillin

O Metronidazole

O Vancomycin

O Zinginezo

SEHEMU YA NNE

DAWA MTAZI

1). Umetumia dawa za kupunguza asidi ya tumbo mwezi umepita?

O Ndio   O La

1b).Kama ndio, ilikuwa gani? …………………….

3).Unatumia dawa ya kutibu saratani?

O Ndio   O La

3b).Kama ndio, sema ni gani? ……………..

4).Unatumia dawa za kupunguza maumivu?

O Ndio   O La

4b). Kama ndio, elezea ni gani? …………………

5). Umetumia dawa za kuachisha kuhara?

O Ndio   O La

5b). Kama ndio, elezea ni gani? ………….

6).Unatumia dawa za kutibu shinikizo la damu?

O Ndio   O La

7). Uko na kisukari na unakunywa dawa zake?

O Ndio   O La

8).Uko na kifua kikuu na unakunywa dawa zake?

O Ndio   O La

SEHEMU YA TANO

MAGONJWA ZINGINE

1).Umekuwa na ugonjwa wowote unaohusu matumbo miezi tatu imepita

O Ndio   O La

1b).Unaweza elezea? ………………………………

2).Uko na saratani ya aina yeyote?

O Ndio   O La

2b).Elezea ni ya kiungo gani ya mwili?

3).Umepimwa ukimwi?

O Niko na ukimwi   O Sina ukimwi   O Sijui

4).Umekuwa na kiungo cha mwili kimepandikizwa?

O Ndio   O La

5).Umeshawahi chinjwa sehemu yoyote ya tumbo?

O Ndio   O La

SEHEMU YA SITA

VIPENGELE ZINGINE

1). Unafuga wanyama kwa nyumba ama kwa shamba?

O Ndio   O La

1b).Kama ndio elezea ni mnyama mgani? ………………..

2). Umeshawahi lazwa kwa hospitali mwaka umepita?

O Ndio   O La

3).Umeshawahi pewa chakula kwa mipira?

O Ndio   O La

4).Unaishi na motto mchanga kwa chumba chako?

O Ndio   O La

APPENDIX 7a- Study Information Sheet (English version)

Study Title: Isolation and Characterization of toxigenic Clostridium difficile among patients attending selected Health Facilities within Nairobi County
I am a student at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID), conducting research on infection with Clostridium difficile which causes diarrhoea within the community
What I will do

You will be given a stool collection clean container, you go to the washroom and put some sample of the diarrhoea and return it to the consultation room. The stool sample will be preserved and transported to the University of Nairobi, Microbiology Department for some laboratory procedures to identify the causative agent of the diarrhoea. The results will be communicated to the clinician for medical advice. In addition, you will be asked a few questions related to the diarrhoea, any other illnesses you have and the medications you might have been taking.

Why I have come to you

Because you have presented to the outpatient department of this health facility complaining of diarrhoea, I need your permission and collaboration to carry out this study. The information generated will be very useful for making decisions about *Clostridium difficile* infection acquired in your community and the country at large.

Risks and benefits

There are no risks involved in participation in this study as no invasive procedures will be carried out. No medication will be administered to enable stool collection from you or your child or children. If you or your child/ children are found infected you will obtain further medical advice from the clinician in this health facility.

Confidentiality

The information documented from this work will not be divulged to anybody and will be used by the study investigator only for purposes of report writing. No information that can identify you will be used in the reports. After reports have been written the information collected from you will be kept private for reference by the investigators only.

Conditions for participation

I have selected you and your child/ children for the study, but the final decision to participate is yours. You are free to accept or reject the participation of you/ your child/ children in the study. If you accept to take part you remain free to withdraw yourself or your child/ children from the study at any time. Your rejection will not affect you or your child/ children access to any public health service.

If you have any questions; I will be readily available to answer any questions OR you may contact me the Principal Investigator on 0729592084 or UNITID on 0716656629 or Chairman, KNH/UoN Ethics Research Committee on Telephone number 020-2716300 Ext 44152 Nairobi.

APPENDIX 7b –Study Information Sheet (Kiswahili version)

"Tabia ya Kliniki na Mikrobiolojia Ya *Clostridium difficile* katika maambukizi kwa makao ya jamii kati ya wagonjwa wanaohudhuria vituo vya afya teule ndani ya kaunti ya Nairobi."

Mimi ni mwanafunzi katika Chuo Kikuu cha Nairobi, Chuo ya kitropiki na kuambukiza magonjwa (UNITID). Nafanya utafiti juu ya maambukizi na *Clostridium difficile* ambayo husababisha kuhara ndani ya jamii katika watu ambao hawajalazwa hospitalini katika miezi 3 iliyopita.

Nitachokifanya

Utapewa kibuyu safi ukusanye kinyesi baadhi ya sampuli ya kuhara na na kurudisha kwenye chumba cha mashauriano. Sampuli ya kinyesi kuhifadhiwa na kusafirishwa kwa Chuo Kikuu cha Nairobi, Idara ya Microbiology kwa taratibu baadhi maabara kutambua wakala wa sababu ya kuhara. Matokeo yatatolewa kwa mhudumu wa afya kwa ushauri wa daktari. Aidha, utaulizwa maswali machache kuhusiana na kuhara, magonjwa mengine ukonayo na dawa wewe ama motto ama watoto wako huenda umekuwa ama wamekuwa kuchukua.

Sababu za kukuchagua

Kwa sababu umejiwasilishwa katika idara ya matibabu ya hiki kituo cha afya na malalamiko ya kuhara, nahitaji yako kibali na kushirikiana kufanya utafiti huu. Taarifa iliyotengenezwa itakuwa muhimu sana kwa ajili ya kufanya maamuzi kuhusu maambukizi ya *Clostridium difficile* katika jamii yako na chi kwa ujumla.

Madhara na manufaa

Hakuna madhara yanayohusika na utafiti huu. Hakuna dawa zitakazopeanwa ili kuwezesha ukusanyaji wa kinyesi kutoka wewe au mtoto au watoto wako. Kama wewe au mtoto wako / watoto patikana kuambukizwa yule mgonjwa atapata ushauri wa matibabu kutoka kwa mhudumu wa afya katika kituo hiki cha afya.

Uhifadhi wa siri

Taarifa ya kumbukumbu kutokana na kazi hii hayatajulishwa kwa mtu mwingine yeyote na kutumiwa na mchunguzi wa kujifunza tu kwa madhumuni ya kuandika ripoti. Hakuna mambo yanayoweza kukuhusisha na utafiti huu yatatumiwa katika kuandika ripoti hio. Baadaye, mambo yatakayo julikana kukuhusu yatahifadhiwa kama siri na mtafiti mkuu peke yake.

Maharti ya kushiriki

Tumekuchagua wewe na motto/ watoto wako lakini uamuzi wa mwisho kushiriki katika utafiti huu ni wako. Uko huru kukubali ama kukataa wewe au motto/ watoto wako kushiriki. Ukikubali, bado utakuwa huru kujiondoa au kuondoa motto/ watotot wako katika utafiti huu wakati wowote. Kukataa au kujiondoa kwako au motto / watoto wako hakutakusababisha adhabu yoyote au kukatazwa huduma katika vituo vya uma vya afya.

Kama uko na maswali; Nitakuwa hapa tayari kujibu swali lolote kuhusu utafiti huu au uwasiliane na mimi 0729592084, chuo changu UNITID 0716656629 au Mwenyekiti, KNH/UoN Ethics Research Committee nambari ya simu 2716300 Ext 44152, Nairobi.

APPENDIX 8a- Informed Consent Form (English version)

I**…………………………………………………………………..**ID NO…………………

From …………………………………………………. (Sub-county and County) being of 18 years or older and having the full legal capacity to consent for myself and my child or children (named below), have been informed about the study entitled:

Title: Isolation and Characterization of toxigenic *Clostridium difficile* among outpatients attending selected health facilities within Nairobi County,

Nature, duration purpose, voluntary nature and inconveniences or hazards that may reasonably be expected have been fully explained to me. I have understood the information regarding the study and what will happen. I have been given the opportunity to ask questions concerning this study and these (if any) have been answered to my satisfaction.

Right to withdrawal

I understand that I may at any time during the study, withdraw the consent in the best interest of myself and that of my children without any loss or penalty. My refusal of the subject to participate will involve no penalty or loss of benefits to which my family or otherwise entitled.

Tick only (√) one box per individual

Participant's name	Age (years)	I **DO** Consent	I **DO NOT** Consent
1).			
2).			

Parent's signature or left thumbprint……………………………….. Date …………………

Witness: I hereby confirm that the study has been explained to the parent. All questions (if any) have also been answered to her / his satisfaction and her / him of her / his own free will, have consented for her/ his child/ children to take part in the study.

Name of witness	
Signature of the witness & Date	

Name of the person explaining the study…………………………………………………………….

APPENDIX 8b –Informed Consent Form (Kiswahili version)

Mimi ……………………………………… Nambari ya kitambulisho ……………..
Kutoka ………………………………. (kijiji na jumbo) kwa kuwa nina umri wa miaka 18 au zaidi na nina uwezo wa kisheria kukubali na kuruhusu mimi na motto / watoto waliotajwa hapo chini, nimeelezewa kuhusu utafiti huu ujulikanao kama:

"Tabia ya Kliniki na Mikrobiolojia Ya *Clostridium difficile* katika maambukizi kwa makao ya jamii kati ya wagonjwa wanaohudhuria vituo vya afya teule ndani ya kaunti ya Nairobi."

Nimeelezewa kwa kikamilifu aina, muda, lengo, hiari ya uhusikaji na madhara yanayo tarajiwa katika utafiti huu. Nimeemlewa maelezo kuhusu utafiti huu na vile utakavyo fanyika. Nimepewa fursa ya kuuliza maswali kuhusu utafiti huu na (kama kunayo) yamejibiwa kwa njia ya kuridhisha.

Haki ya Kujiondoa kutoka utafiti huu
Ninaelewa kwamba ninaweza kuondoa kibali changu au cha motto/ watoto wangu kuhusika katika utafiti huu wakati wowote bila adhabu au hasara yoyote. Kukataa kwangu au mtoto/ watoto wangu kuhusika katika utafiti huu hakutasababisha adhabu yoyote ya kupoteza manufaa yoyote ambayo jamii yangu inastahili

Weka alama (√) katika sanduku

Jina la muhusika	Umri (miaka)	Nakubali	Nakataa
1).			
2).			
3).			

Sahihi au alama ya kidole guba cha mzazi muhusika ……………… Tarehe ……….

Shahidi: Ninadhibitisha ya kwamba mzazi/ mlezi ameelezewa kuhusu utafiti huu. Ameridhishwa na majibu aliyopewa kwa maswali yote (kama anayo) na kwa hiari yake yeye mwenyewe amekubali mtoto/ watoto wake wahusike katika utafiti huu

Jina la shahidi	
Sahihi ya shahaidi Tarehe	

Jina la anaye eleza kuhusu utafiti huu ………………………………………………………

APPENDIX 9a- Minor Assent Form (English version)

I Ruth Wandia Maina a student at UNITID, am doing a research on diarrhoea within the community settings caused by *Clostridium difficile.*

The study title is **"Isolation and characterization of toxigenic Clostridium difficile among outpatients in selected health facilities within Nairobi County."**

Permission has been sought from the KNH/ UON Ethics Research Committee.

This research aims to learn how diarrhoea within homesteads can be prevented by knowing the risk factors and how to mitigate them.

Other children like you will be participating and all is needed is a collection of your stool sample with no invasive procedures involved. You will not undergo any painful procedures.

If you decide to participate in this study you will be given a clean sample collection container and asked to bring in a stool sample to the consultation room. The sample will be preserved and transported to the UoN Microbiology department for some laboratory procedures to determine the causative agent of the diarrhoea and if found infected medical advice will be offered accordingly.

You can refuse participation voluntarily and you can still opt out even after the study has begun. Your parents know about the study too.

If you decide you want to participate please sign your name below

I ………………………………………… want to be in this research study.

---   Date --------------------------------

(Signature/ thumb stamp)

APPENDIX 9a –Minor Assent Form (Kiswahili Version)

Naitwa ………………………………………….

Nasomea chuo kikuu cha Nairobi (UNITID).

Nafanya utafiti wa usababishaji wa kuhara katika maeneo ya jamii unaohusika na *Clostridium difficile.*

Huu utafiti unaitwa ""**Tabia ya Kliniki na Mikrobiolojia Ya *Clostridium difficile* katika maambukizi kwa makao ya jamii kati ya wagonjwa wanaohudhuria vituo vya afya teule ndani ya kaunti ya Nairobi."**

Nimepewa kibali na KNH/UON Ethics and Research Committtee wanaosimamia maadili ya utafiti Kenya. Utafiti huu unakusudia kujifunza jinsi kuhara katika maeneo ya jamii inaweza zuuliwa.

Watoto wengine kama wewe wanahusika na kinachohitajika ni upewe kibuyu safi, uweke baadhi ya kinyesi cha mharo kwa hicho kibuyu, rudisha kwa chumba cha mawasiliano. Kinyesi kitahifadhiwa na kupelekwa kwa maabara na ukipatikana na maambukizi ya *Clostridium difficile* ushauri utatolewa ipasavyo.

Unaweza kataa kushiriki kwa hiari na unaweza bado kujiondoa hata baada ya utafiti umeanza. Wazazi wako wamejulishwa kuhusu huu utafiti.

Kama umekubali kushiriki tafadhali weka jina au sahihi hapa chini.

……………………………………….   Tarehe ………………………

(Sahihi/ Kidole)

Hakuna taratibu vaamizi au maumivu yeyote kushiriki.

APPENDIX 10- Quality Assurance Protocol

University of Nairobi Department of Medical Microbiology Quality Assurance Protocol for Real Time PCR Analysis.

This QA management Plan describes the routine/ day –day operations in the Laboratory to ensure quality and reproducibility of results. It describes the basic requirements for the Personnel, Facility, Samples handling and the Sampling procedures, Quality checks needed for equipment maintenance, Laboratory supplies and Reagents as well as procedures for maintaining cleanliness.

Quality Assurance

In this study the following steps will be followed to ensure that there is control of errors in the performance of tests and verification of test results.

There will be adequate control of materials, equipment and procedures.

    a) Sample collection

Freshly collected in a clean, dry, air-tight, leak-proof and dry wide- mouthed sample container. The sample will not be contaminated with water and urine as the study nurse will guide the patient on this in addition to collecting at least 15mls of the sample.

The patient will wash hands before and after collecting the sample with clean water and soap.

    b) Transportation and packaging of the sample

Upon receipt of the sample in the sample container, the study nurse will attach a label indicating the name of the patient, the health facility, date and time of collection, the colour and consistency of the specimen whilst ensuring that the container is leak- proof. This will be stored in a cool box whose temperature will be monitored and maintained at 2-4º C under which conditions the fecal sample will be transported to the ISO certified UoN Department of Microbiology laboratory .

    c) Sample processing

Receipt in the laboratory will be by a trained personnel who will check to ensure the sample is well labeled, sufficient in quantity and well preserved. Then consecutive study procedures will be carried out using commercially prepared and quality certified reagents, supplements and culture media.

    d) Internal Quality assurance in the laboratory

To validate the results, different trained personnel will be required to pick a few already tested samples and subject them to the same procedures to ensure reproducibility of results and if not then corrective action plan used by the UoN microbiology laboratory will be followed.

Section 1 –Personnel

1.1 Background and Training requirements

Undergraduate training in course work that covers PCR and recombinant DNA theory and practice like molecular biology and biochemistry.

Supervised applied training like the review of SOPs for each applicable laboratory.

Demonstrated successful capability to perform analytical procedures under minimal or no supervision.

Retention of training records that is inclusive of;

- o The dates of specific and the range of PCR training

- o Proficiency tests results for each analytical method

- o The dates and range of QA/QC laboratory training

- o The dates and the range of safety training for the laboratory

- o Initial demonstration of successful analytical capability

1.2) Outerwear

I.   Laboratory coats should be removed and gloves discarded before leaving each room

II.   Dedicated laboratory coats and powder-free gloves should be available at all times

III. Laboratory coats should be cleaned regularly to reduce the possibility of contamination of the designated workspace and the PCR reaction

IV.   Disposable laboratory coats also may be used.

Section 2- Facility Design and Workflow

The laboratory should be designed and operated in a way that prevents contamination of reactions with amplified products from previous assays and cross-contamination between samples, both of which can lead to false-positive results.
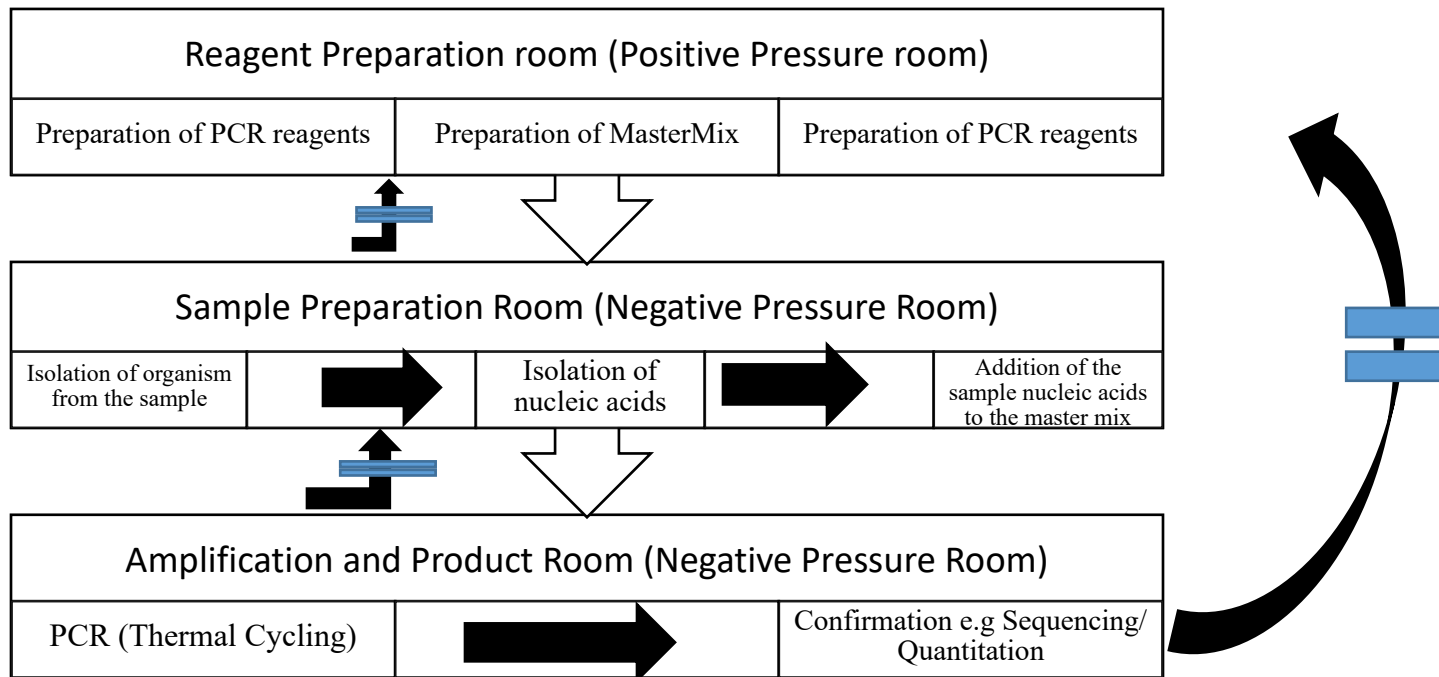
2.1 Facility design

The laboratory has a unidirectional workflow and has been divided into three physically separate rooms to reduce the chances of contamination namely;

- o Reagent preparation room,

- o Sample preparation room

- o Amplification and product detection rooms

Separate biological safety cabinets are dedicated for positive control and processing of test sample(s).

Vacant areas in the laboratory have a unidirectional flow and dedicated for non-PCR activities. The equipment is not to be moved between the PCR sample processing rooms and analytical procedures.

Reagent Preparation room (Positive Pressure room)		
Preparation of PCR reagents	Preparation of MasterMix	Preparation of PCR reagents

Sample Preparation Room (Negative Pressure Room)		
Isolation of organism from the sample	Isolation of nucleic acids	Addition of the sample nucleic acids to the master mix

Amplification and Product Room (Negative Pressure Room)	
PCR (Thermal Cycling)	Confirmation e.g Sequencing/ Quantitation

2.2 Reagent Preparation Room

Designated for sample preparation and storage of PCR agents inclusive of Master mixes

Dedicated pipettes with plugged positive displacement pipette tips, dedicated laboratory coats and dedicated disposable gloves.

2.3 Sample Preparation Room

Designated for sample processing and preparation of positive and negative controls.

Processing of samples includes concentrating of target organisms in environmental samples plus extraction and purification of nucleic acids from the organisms; then the processed samples and controls are added to the tubes with PCR Master Mix here.

In this room there are;

- o Dedicated adjustable pipettes with plugged, aerosol-barrier tips or positive-displacement tips, dedicated fresh gloves and laboratory coats

- o Two biological safety cabinets

- o Each hood has dedicated pipettes and laboratory coats.

- o A designated enclosed area with dedicated refrigerators for sample receiving and sample storage.

2.4 Amplification and Product room

Designated for activities associated with PCR amplification and Post-PCR analyses and has the following equipment;

- o The thermocycler

- o Dedicated gloves and laboratory coats

- o All dedicated equipment for amplification and product detection.

- o Dedicated adjustable pipettes with plugged, aerosol-barrier or positive displacement pipette tips.

2.5 Environmental Sample acceptance

The sample is assessed upon receipt in the laboratory to verify that the sample volume is adequate, the sample has been handled and appropriately preserved, the holding time requirement has been met, and that all required sample collection information has been recorded by the sample collector. After the assessment, the date and time of sample receipt and the condition of the sample is recorded whilst using a unique identifier the sample is marked, logged and tracked.

Section 3- Equipment

They are in a dedicated laboratory room and the schedule includes the setup, calibration, repair, records keeping, and conventional operation of all analytical equipment.

3.1 Thermocyclers and real time PCR instruments

The block temperature of the thermocycler is tested at least twice annually. This is done using an external probe that has been calibrated against a temperature standard.

Real time PCR instruments performance, alignment, and safety devices are checked and optical systems calibrated once annually as per the manufacturers specifications.

3.2 Centrifuges

Used for pre and post-PCR procedures and calibrated before a batch of samples are run.

3.3 Gel Electrophoresis chambers

Chambers are inspected before each use to ensure that electrodes and buffer tanks are intact and that power supply electrodes fit snugly. The chambers are rinsed several times with water after each use in the designated product room.

3.4 Hybridization Apparatuses

General maintenance entails cleaning of the incubator, with distilled water periodically, usually twice annually for as well as the temperature and the rotation/ rocking speed of the oven so as to match the registered values indicated on the unit.

3.5 Sequencers

Preventive maintenance is done annually

3.6 Biological safety cabinets/ Laminar – flow hoods

Before use the hoods are decontaminated using UV light for at least 8 hours and cleaned with bleach. Annually, the airflow and HEPA filtration in all hoods, is monitored and certified following the manufacturer's recommendations.

3.7 Ultraviolet lights

The UV bulb is wiped with a wet cloth to remove dust every week. The UV lights are checked for intensity loss using a UV light meter once a month.

3.8 Pipettes

They are calibrated quarterly using the tips commonly used in the laboratory or whenever contamination is suspected .Calibration is after sterilization.

3.9 Temperature Dependent equipment

Calibration of thermometers is once annually.

For equipment used in PCR analysis, the following temperature ranges are applied:

i. Incubators, water baths, and heating blocks: $\pm$ 0.5 °C of the temperature required by the protocol

ii. Refrigerators: 1°C to 5°C

iii. Standard laboratory freezers: -20°C $\pm$ 5°C

iv. Ultra-low freezers: -70°C $\pm$ 10°C

3.10 Spectrophotometers, Luminometers, and Fluorimeters

Fluorescent detectors are calibrated every three to six months or more frequently if a problem is detected. Calibration is carried out through spectral calibration solutions needed to establish the pure dye spectra. These dyes are of known spectra and come with a normalization reference. Standard light plates and tubes can be obtained from the manufacturer for the calibration of luminometers that allow the reproducibility, sensitivity, and linearity of the luminometer to be confirmed.

3.11 Disposables

Pipette tips, gloves and sample and PCR tubes are properly disposed to avoid contamination.

Section 4- Laboratory cleaning

All work surfaces are cleaned after each use with 0.6% sodium hypochlorite (NaOCl) prepared fresh daily. Thermocyclers and centrifuges are cleaned with the diluted bleach solution whenever contamination is suspected. Pipettes too are cleaned as per manufacturers' instructions. Racks and trays should are soaked in the 0.6% NaOCl solution and thoroughly rinsed with water after each use. Gel-trays, gel combs, and glassware are rinsed with water or a mild detergent after each use.

Section 5- Reagents

They are bought and they are clearly labeled with name, expiration date, and relevant safety information whilst avoiding an interchange.

5.1 Primer sets and hybridization Probes

Commercially bought and come with a certification of analysis. Purity is assessed by HPLC or by separation on an acrylamide gel of appropriate concentration. Functional validation is also

performed on new lots of primers and probes by comparing their performance against older sets of known quality.
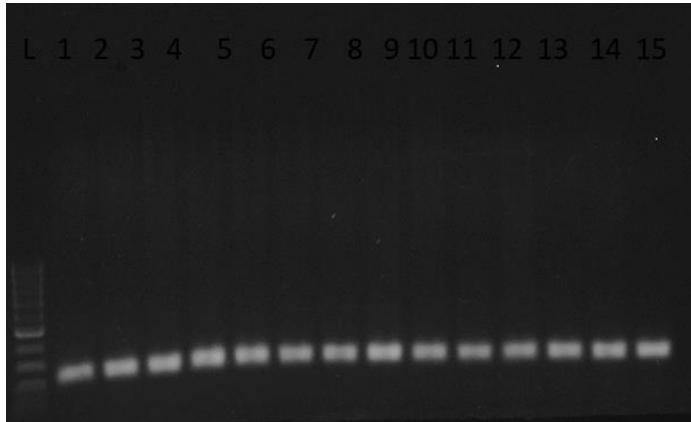
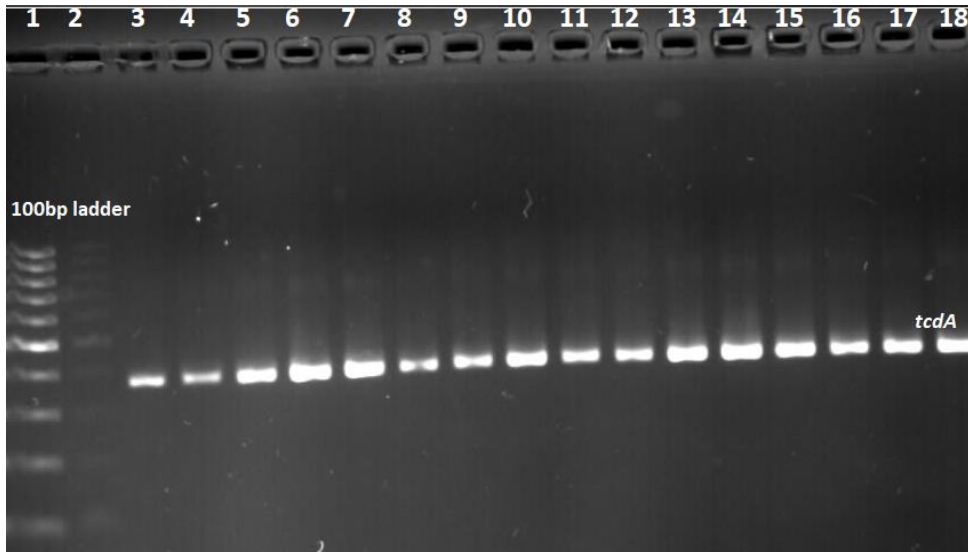**APPENDIX 11- Morphological identification of Clostridium difficile under an ultraviolet light**
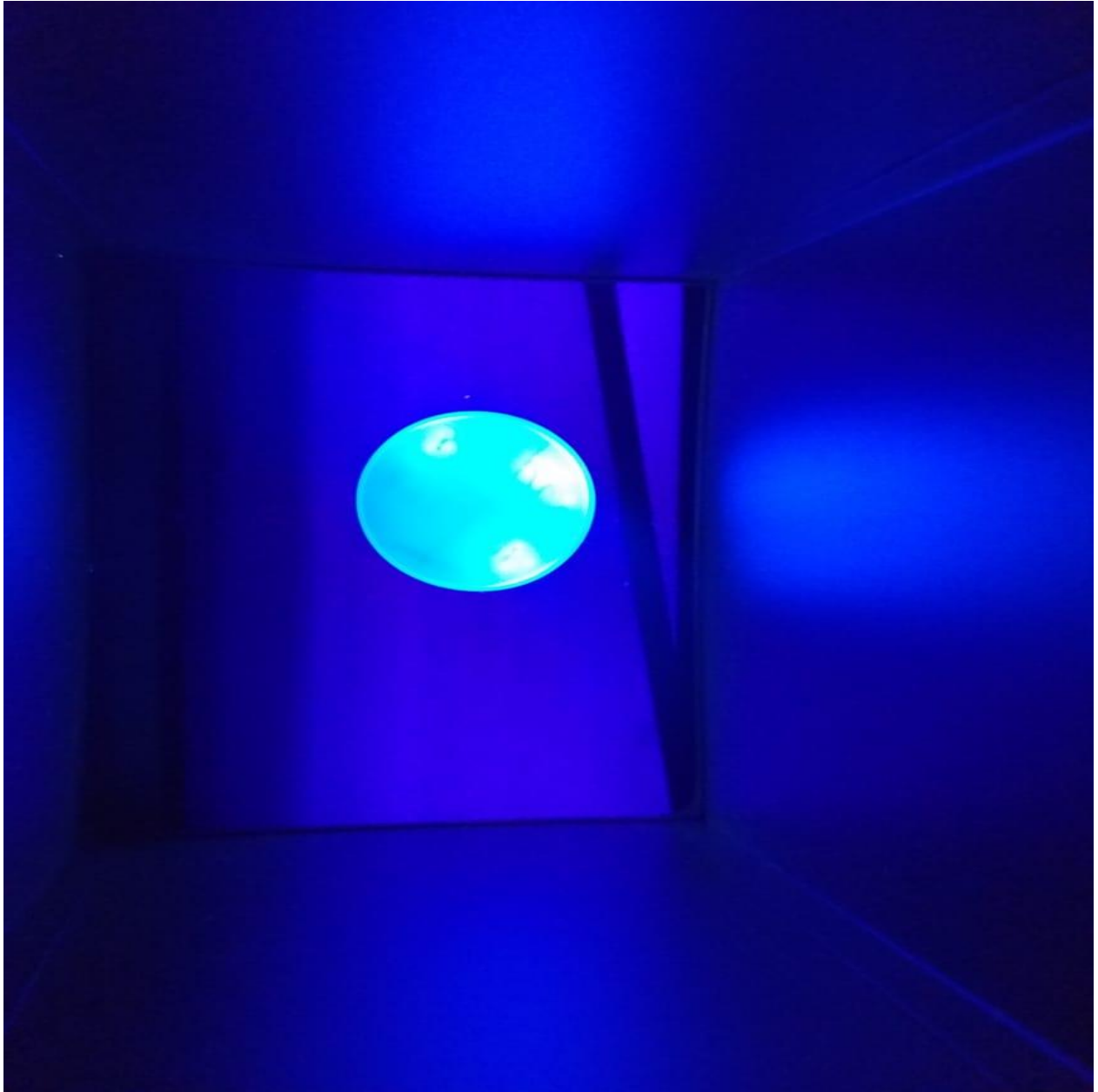
Fluorescence of Clostridium difficile colonies on CHROMagar and observed under an ultraviolet light.
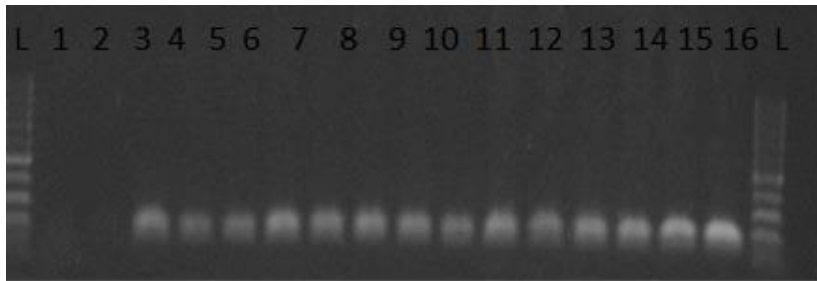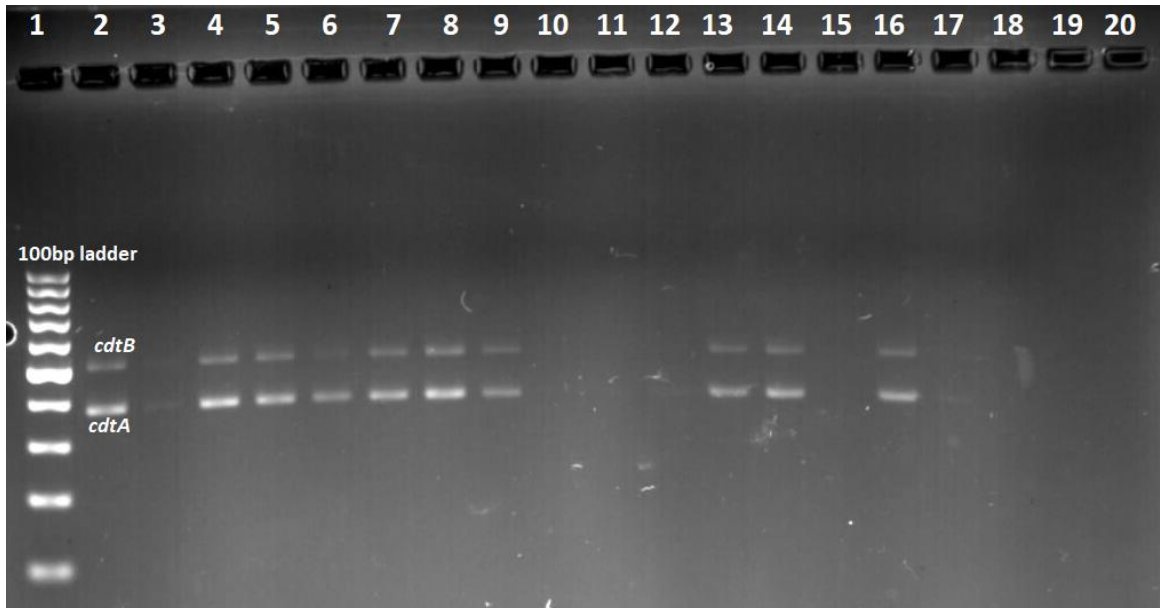
APPENDIX 12 –Toxin genes PCR bands

Lane L: 100bp ladder; Lane 1-15: clinical sample with tpi genes (230bp)

Lane 1: 100bp ladder; Lane 2: Negative control; Lane 3: Positive control; Ladder 4-18: tcdA positive clinical samples

Lane L: 100bp ladder; Lane 2: Negative control; Lane 3: Positive control (160bp); Lane 4-17 tcdB+ clinical samples; Lane 18; Negative control

Lane 1: 100bp ladder; Lane 2: Positive control; Lane 3-9, 13-14, and 16: Clinical samples with binary toxin (cdtA and cdtB); Lane 10-12, 15, 17-19: Clinical samples without binary toxin; Ladder 20: Negative control

Stool samples