PREVALENCE OF CLOSTRIDIUM DIFFICILE INFECTION IN HOSPITAL SUNGAI BULOH AND THE ASSOCIATED RISK FACTORS

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ABSTRACT

Clostridium difficile infection (CDI) is the major recognized cause of nosocomial diarrhoea. It causes mild to severe diarrhoea and also pseudomembranous colitis. One of the most important risk factors for CDI is prior antibiotic exposure. Data on CDI among Malaysian patients are still very limited. We aim to look at the prevalence of CDI among inpatients of Hospital Sungai Buloh. This study involved data collection of the stool specimens from 198 patients suspected to have CDI. The presence of Toxin A and Toxin B was tested by a commercial immunochromatographic rapid test kit (Duo Toxin A+B – Check-1 by VEDALAB-France), followed by culture for *C. difficile*. Secondary data were collected from the patients' medical records to determine demographic data, clinical presentations and associated risk factors. The prevalence of CDI among our patients was 6.1%, and majority of the patients were male. The most common presentations, apart from diarrhoea, were fever, abdominal pain and nausea. Leucocytosis, dehydration and electrolyte imbalances were the common signs. The median length of stay was 16 days. The most common preceding antibiotic was amoxycillin. All of our patients were nursed in double or more occupancy room. Eight (66.7%) had history of admission to long-term healthcare facilities within the past 1 year and 10 (83.3%) of the CDI patients were hypoalbuminaemic. In conclusion, the prevalence of CDI in Hospital Sungai Buloh is relatively low.

ABSTRAK

Jangkitan *Clostridium difficile* (CDI) adalah antara penyebab utama cirit-birit yang dikaitkan dengan jangkitan yang diperoleh semasa seseorang menerima rawatan di fasiliti kesihatan. Penyakit ini boleh menyebabkan cirit-birit ringan hingga berat, dan juga kolitis pseudomembran. Kajian ini dijalankan untuk menentukan prevalen CDI di kalangan pesakit yang dimasukkan ke Hospital Sungai Buloh. Kajian ini melibatkan pengumpulan data daripada 198 pesakit yang disyaki mempunyai CDI. Kewujudan toksin A dan B diuji dengan kit imunokromatografi komersial (Duo Toxin A+B – Check-1 oleh VEDALAB-France), dan seterusnya kultur dilakukan untuk penumbuhan *C. difficile*. Data sekunder dikumpul daripada rekod pesakit untuk mengenalpasti ciri-ciri demografi, klinikal dan faktor risiko untuk CDI. Prevalen CDI di kalangan pesakit di Hospital Sungai Buloh adalah 6.1%, dan majoriti pesakit adalah lelaki. Ciri klinikal utama untuk CDI, selain daripada cirit-birit, adalah demam, sakit perut, loya, ketidakseimbangan garam galian dalam darah, dehidrasi dan peningkatan bilangan sel darah putih. Median tempoh inap di hospital adalah 16 hari. Antibiotik yang paling kerap dikaitkan dengan CDI di kalangan pesakit kami adalah *amoxycillin*. Semua pesakit CDI kami terdiri daripada mereka yang dimasukkan ke bilik untuk dua atau lebih pesakit. Lapan (66.7%) daripada mereka mempunyai sejarah kemasukan ke fasiliti kesihatan jangka panjang dalam masa 1 tahun yang lepas, dan 10 (83.3%) mempunyai paras albumin darah yang rendah. Secara rumusannya, prevalen CDI di kalangan pesakit yang dimasukkan ke Hospital Sungai Buloh adalah rendah.

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LIST OF SYMBOLS AND ABBREVIATIONS

AAC	:	Antibiotic-associated colitis
AAD	:	Antibiotic-associated diarrhoea
AST	:	Antibiotic susceptibility testing
CCFA	:	Cycloserine-cefoxitin, fructose agar
CCNA	:	Cell cytotoxicity neutralization assay
CDA	:	Clostridium difficile agar
CDAD	:	Clostridium difficile associated diarrhoea
CDI	:	Clostridium difficile infection
EIA	:	Enzyme immunoassay
GDH	:	Glutamate dehydrogenase
HCW	:	Healthcare worker
HIV	:	Human Immunodeficiency Virus
HSB	:	Hospital Sungai Buloh
HUSM	:	Hospital Universiti Sains Malaysia
IBD	:	Inflammatory bowel disease
ICAAC	:	Annual Interscience Conference on Antimicrobial Agents and Chemotherapy
ICU	:	Intensive care unit
IDSA	:	Infectious disease society of America
PCR	:	Polymerase chain reaction
PEA	:	Phenyl-ethyl alcohol
PMC	:	Pseudomembranous colitis
SHEA	:	Society for Healthcare Epidemiology of America

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

Other than development of resistance, one of the major disadvantages of antibiotics administration is *Clostridium difficile* infection (CDI). CDI is a major recognized cause of nosocomial diarrhoea. Antibiotics commonly associated with colonization of the intestines with *C. difficile* are clindamycins, cephalosporins and ampicillin (Bélanger, Boissinot, Clairoux, Picard, & Bergeron, 2003). *Clostridium difficile* colitis results from disturbances of normal flora of the colon, colonization by *C. difficile* and the release of toxin that cause mucosal inflammation and damage.

The link between antibiotic and pseudomembranous colitis was described since the 1950s. *Staphylococcus aureus* was presumed to be the pathogen involved. However, another existing pathogen was implied in view of increasing reports implicating association of clindamycin – an excellent anti-staphylococcal agent – and pseudomembranous colitis (Tedesco, Barton, & ALPERS, 1974). In 1978, a study showed links between *Clostridium difficile* with pseudomembranous colitis in humans and hamsters (Larson, Price, Honour, & Borriello, 1978). The incidence of *C. difficile* diarrhoea has increased since the 1980s. Recent reports suggest the emergence of a new epidemic virulent *C. difficile* strain (type 027) in Canada, USA and, most recently, the UK. A Singapore study by XQ Tan et al in 2014 showed the prevalence of CDI in Singapore of 10.7/10 000 patient days or 6.38/1000 discharges (Tan et al., 2014).

Clostridium difficile infection is generally underdiagnosed in the community setting. A Swedish study found that 42% of cases of *C. difficile* infection presented in the community, and half of these did not have a history of hospitalisation within the previous month (Karlström, Fryklund, Tullus, & Burman, 1998). A study done in Singapore revealed that 50% of CDI is community onset, and of these, 27.3% were community associated (Tan et al., 2014) and ribotyping of the isolates revealed that the predominant

strains being O53 and O12. Evidently, the distinction between nosocomial and community-acquired *C. difficile* diarrhoea is blurred by the overlap between primary and secondary healthcare provision, as more healthcare activities shift to outpatient settings.

Essentially, control of CDI is the control of antibiotic prescription. However, despite the link between antibiotic prescribing and CDI, the incidence of CDI continues to increase. Spread of nosocomial CDI is believed to occur via hands of healthcare workers and contaminated environment, and possibly direct patient to patient spread. Infected patients should be isolated or cohort nursed. Hand hygiene should be emphasized to the patients, care-takers and visitors, and healthcare workers.

This research project is carried out to determine the prevalence of CDI in Hospital Sungai Buloh. In addition, it is hoped that this study will provide data on primary risk factors and the antibiotics associated with *Clostridium difficile* infection amongst the inpatients admitted to Hospital Sungai Buloh. These data can be used to instil awareness among clinicians and encourage them to practice antimicrobial stewardship in managing their patients. Lastly, this study sought to determine the association between clinical presentations of Clostridium difficile infection to the detection of *Clostridium difficile* by routine microbiological investigations.

1.2 RESEARCH QUESTIONS

i. What is the prevalence of *Clostridium difficile* in Hospital Sungai Buloh and the antimicrobial susceptibility pattern?

ii. Who are at risk to develop *Clostridium difficile* infection?

iii. What are the antibiotics used in Hospital Sungai Buloh that are commonly associated with *Clostridium difficile* infection?

iv. Is there any association between the clinical features of CDI and the microbiological detection of the organism?

1.3 OBJECTIVES

1.3.1 PRIMARY OBJECTIVE

To determine the prevalence of *Clostridium difficile* infection in Hospital Sungai Buloh and the antimicrobial susceptibility.

1.3.2 SECONDARY OBJECTIVES

i. To study the primary risk factors for developing *Clostridium difficile* infection amongst the inpatients admitted to Hospital Sungai Buloh.

ii. To identify the antibiotics associated with *Clostridium difficile* infection in Hospital Sungai Buloh.

CHAPTER 2: LITERATURE REVIEW

Clostridium difficile is an anaerobic spore-forming gram positive rod and it is the most common nosocomial cause of diarrhoea. First isolated in 1935, it was believed to be non-pathogenic for humans until late 1970s, when it was implicated as a causative agent in antibiotic-associated diarrhoea (AAD) and pseudomembranous colitis (PMC) (Winn & Koneman, 2006). Approximately 3% of the general population carry this organism in their gastrointestinal tract, and the percentage increases up to 30% in hospitalized patients.

Hospitalized patients that are treated with antibiotics may develop benign, self-limiting diarrhoea and this diarrhoea usually resolves after discontinuation of antibiotics. In most cases, the cause of the diarrhoea is not determined. However, some patients may have more severe symptoms. These patients may have antibiotic associated colitis (AAC) or PMC. The most common cause of PMC possibly is *Clostridium difficile*. Other causes include *Staphylococcus aureus* or *Clostridium perfringens* (Winn & Koneman, 2006). Besides PMC, *Clostridium difficile* is associated with about 60 to 75% of AAC and 11 to 33% cases of AAD (McFarland, Mulligan, Kwok, & Stamm, 1989). Factors linked to *Clostridium difficile* infection include:

a. Toxin A, which is an enterotoxin
b. Toxin B, which is a potent cytotoxin
c. A 'motility altering factor' which stimulates smooth muscle contractions of intestine

Clostridium difficile has been isolated from soil, water, intestinal contents of various animals, vagina and urethra of humans, and faeces of many healthy infants. Although it is found in only about 3% of the stools of healthy adults, this organism is found in the faeces of up to 13 to 30% of hospitalized adults despite being asymptomatic for *Clostridium difficile* associated disease.

Antibiotics that are linked to CDI include aminoglycosides, penicillins, cephalosporins, second- and third-generation beta lactam compounds, clindamycin, erythromycin, lincomycin, metronidazole, rifampin, trimethoprim-sulfamethoxazole, amphotericin B and the fluoroquinolones (Gerding, 2004; Gerding, Johnson, Peterson, Mulligan, & Silva, 1995).

For laboratory diagnosis of *Clostridium difficile*, liquid or semisolid, unformed faecal specimens are the preferred specimens. Other suitable specimens include biopsy material or lumen contents obtained by colonoscopy and involved bowel (surgical removal; autopsy). The specimens should be sealed in a plastic container during transportation. The specimen can be transported to the laboratory in room temperature provided it is sent on the same day as sampling. In the event of delay is unavoidable, the samples can be refrigerated. Specimens that are sent to reference laboratory for toxin assays are to be transported on dry ice, whereas those sent for isolation and identification of *Clostridium difficile* should be transported anaerobically at 25°C (Winn & Koneman, 2006). Specimens to be processed for *C. difficile* latex agglutination test should not be frozen because the antigen detected is unstable in freezing state.

The diagnosis of CDI is based on either demonstration of cytotoxin, by isolation and identification of *Clostridium difficile* from stool specimens, by performing latex agglutination test or enzyme immunoassay to detect glutamate dehydrogenase of *C. difficile*, by enzyme immunoassays to detect enterotoxin or cytotoxin in faeces, or by combination of methods. Specifically, the diagnosis of CDI is defined by the presence of symptoms (usually diarrhea) and either a stool test positive for *C. difficile* toxins or toxigenic *C. difficile,* or colonoscopic or histopathologic findings revealing pseudomembranous colitis (Cohen et al., 2010). The presence of *C. difficile* toxin is essential to the diagnosis, and absence or negative toxin testing usually makes the diagnosis of CDI unlikely. This is largely due to many studies have shown that

Clostridium difficile carriers do occur. The American College of Gastroenterology recommends that nucleic acid amplification testing (NAAT) for detection of *Clostridium difficile* toxin to be used as standard diagnostic test for CDI (Surawicz et al., 2013). The guideline also stated that NAAT is a good stand-alone test for the diagnosis of CDI. Glutamate dehydrogenase (GDH) screening tests for *C. difficile* can be used in two- or three-step algorithms with subsequent toxin A + B EIA testing, but the sensitivity of such strategies is lower than NAATs. In contrast, the updated Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) guideline on diagnosis and reporting of *Clostridium difficile* has suggested that organisations adhere to a two-stage testing approach which consists of a GDH EIA (or a NAAT or PCR) test to screen samples, followed by a sensitive toxin EIA test (or a cytotoxin assay). If the first test (GDH or NAAT) is negative, the second test (sensitive toxin EIA) does not need to be performed (United Kingdom, Department of Health, 2012).

Clostridium difficile can be isolated by inoculation onto selective media such as phenyl-ethyl alcohol (PEA) blood agar or cycloserine-cefoxitin, egg-yolk, fructose agar (CCFA) or nonselective anaerobe blood agar. The inoculated media is then incubated anaerobically. After 48 hours, colonies of *C. difficile* growing on the selective media cycloserine-cefoxitin fructose agar (CCFA) are approximately 4mm in diameter, yellow ground glass like and slightly filamentous at the edges. The colonies have characteristic odour described as "horse manure" odour. As the colonies ages and sporulates, they become whiter. On anaerobe blood agar, at 48 hours after incubation, colonies are non-hemolytic, about 2 to 4mm on diameter, greyish to translucent, slightly raised flat and spreading, with rhizoid margins. The distinctive odour can also be appreciated here. When viewed under dissecting microscope, the colonies show an iridescent speckled-opalescent appearance. *C. difficile* is negative for lipase and lecithinase on egg yolk agar. The gram stain shows sub terminal spores and the organisms are motile in early broth cultures.

Clostridium difficile hydrolyzes esculin and gelatin, and negative for indole, nitrate and urease. They ferment glucose, mannitol and mannose.

Clostridium difficile infection is widely underdiagnosed. In a study reported in Lancet Infectious Disease, most hospitals were not using recommended methods to test for CDI. The study was done involving 482 hospitals in 20 countries, and it revealed the rate of under diagnosis of CDI ranges from 0% in Belgium, Ireland, the Netherlands, Slovenia and Sweden to over 60% in Bulgaria, Greece and Romania. From the study, amongst the contributing factor to under diagnosis of CDI is lack of standardized system to test for *Clostridium difficile* (Davies et al., 2014). In many countries, test for *C. difficile* is not done if the clinicians do not request for the test. Hence, awareness amongst clinicians and clinical microbiologists is one of the issues that need to be addressed if we aim to reduce the rate of CDI.

In order to have accurate diagnosis for *Clostridium difficile* infection, accurate diagnostic methods are essential. At present, many rapid test kits are being developed aimed at replacing standard reference methods, which are cell cytotoxicity assay and toxigenic culture. Both of the latter techniques are laborious, time consuming and technically demanding. Polymerase chain reaction may give rapid results; however, in the absence of symptoms, false positive results may occur and hence over diagnosis of CDI.

The prevalence of CDI varies with populations. Over the years, the prevalence of CDI is increasing. In the United States, CDI affects over three million diarrhoeal and colitis patients annually (Schroeder, 2005). In Korea, a study done in 17 tertiary hospitals from 2004 to 2008 found that the incidence of CDI increased from 1.7/1000 to 2.7/1000 adult admissions. In Japan, China and Taiwan however, the true incidence of CDI cannot be defined (Collins, Hawkey, & Riley, 2013).

In Malaysia, reports on *Clostridium difficile* infections are rare. A study done in Hospital Universiti Sains Malaysia (HUSM) in 2012 revealed the prevalence of CDI of 13.7% of the stool samples, with majority of the patients affected were aged more than 50 years (Hassan et al., 2012). A more recent study done in Hospital Universiti Kebangsaan Malaysia in 2013 showed the prevalence of CDI is 6.1% (NA & TZAR, 2013). At present, there is no established data for Hospital Sungai Buloh, an infectious disease reference hospital in Malaysia. For this reason, the study is done to determine the prevalence, incidence and clinico-epidemiological characteristics among diarrhoeal patients in Hospital Sungai Buloh.

As aforementioned, *Clostridium difficile* colitis results from colonization of the colon by *Clostridium difficile*. These colonizers release toxins that cause mucosal inflammation and damage. The organisms are most commonly hospital acquired, but there are 3% of populations are carriers, and *Clostridium difficile* is widely found in the environment. A single most important factor in aid of colonization by *Clostridium difficile* appears to be the imbalance of colonic normal flora due to the effect of antimicrobials, which is dependent on agent, route of administration, duration, and host factors. *Clostridium difficile* strains adhere to colonic enterocytes and subsequently start producing toxins. There are 2 recognized *Clostridium difficile* toxins at present, namely toxin A and toxin B. These toxins alter normal cellular physiology, cause distortion in the cytoskeleton and disrupt tight junction leading to inflammatory response with polymorphonuclear aggregates and, in advanced stages, the characteristic pseudomembrane (Voth & Ballard, 2005). Initial studies suggested toxin A was an ''enterotoxin'' implying a major role in causing colonic pathology, whereas Toxin B was initially designated ''cytotoxin'' as it is about 1000 times more potent than toxin A in tissue culture assays. Later studies show *Clostridium difficile* strains that produce toxin B but not toxin A (toxin A–B+) have been reported from many countries, and these strains may also cause disease.

Three major risk factors associated with CDI are prolonged hospitalization, changing patterns of antibiotics use, and aging population (Bignardi, 1998). There is evidence for higher risk for CDI in individuals aged more than 64 years old. Other risk factors are as the followings (Bassetti, Villa, Pecori, Arzese, & Wilcox, 2012; Bignardi, 1998; Moyer, 2006; Stanley, Bartlett, Dart, & Ashcraft, 2013):

a. Female gender
b. Admission to double occupancy rooms
c. Intensive Care Unit (ICU) admission
d. Admission to a long-term care facility within the last year
e. Post-pyloric tube feedings
f. Acid reducing therapy
g. Gastrointestinal procedures
h. Hypoalbuminemia
i. Renal disorders
j. Organ transplantation
k. Human Immunodeficiency Virus (HIV) infection
l. Autoimmune disease
m. Malignancy or chemotherapy
n. Inflammatory bowel disease
o. Smoking

Hospitalization provides both a reservoir for infection and also aid in transmission. Carriers and patients with *Clostridium difficile* colonization provide source new infection and colonization, and the hospital setting is in such a way that control is difficult. Patients may acquire CDI through ingestion of the spores which may be transported from patient to patient by caregivers or from equipment and surfaces.

Preceding antibiotics use is another established risk factors for CDI. Bignardi reported in his study in 1998, all antibiotics including metronidazole are implicated in the development of CDI (Bignardi, 1998). Fluoroquinolones and cephalosporins are at present commonly associated with CDI. In addition, concurrent use of antibiotics is also associated with increased risk for CDI and continuation of antibiotics after diagnosis is associated with recurrence of CDI. Antibiotics cause a loss in commensal bacteria in the colon and as a consequence increase susceptibility for pathogenic species. Molecular analysis on stool samples collected on patients who developed antibiotic-associated diarrhoea suggested that antibiotics lead to changes in faecal bacteriology which may allow overgrowth of Clostridium species and increased susceptibility of the mucosa to toxin effects. Another study reported that oral antibiotics in preparation for intestinal surgery increase the risk for post-operative CDI (Wren, Ahmed, Jamal, & Safadi, 2005). Patients are more likely to acquire CDI if they take imipenem, ceftazidime, clindamycin, or moxifloxacin (Moyer, 2006).

The principal mode of transmission of *Clostridium difficile* is via person-to-person, through faecal-oral route. Other means of transmission include contamination of the hands of healthcare workers (HCW) by *Clostridium difficile* spores, and environmental contaminations. *Clostridium difficile* spores are highly resistant to desiccations, chemicals and extreme temperatures. Spores commonly contaminate the environment of patients with CDI and may persist up to several months or years. The organism is ingested either as the vegetative form or as spores. When the conditions are suitable, these spores will germinate and multiply. The organism, by means of flagella and production of proteolytic enzymes, can then adhere and penetrate the mucus layer that covers the enterocytes. Through their adhesins, *Clostridium difficile* can now adhere to cells and colonize the gut. Subsequently, they start producing toxins A and B, the two main virulence factors. These toxins cause the release of cytokines, which attract

inflammatory cells and promote fluid secretion, resulting in the inflammation of the colon.

The cardinal feature of CDI is watery diarrhoea. However, the clinical manifestations range from being asymptomatic carriers to severe, fulminant toxic megacolon. Symptoms usually begin 5 to 10 days after antibiotic therapy, and sometimes up to 10 weeks after cessation of therapy (T. J. Lamont, 2016; Torok, Moran, & Cooke, 2009). Features include fever, abdominal pain, nausea, dehydration and leukocytosis. *Clostridium difficile* associated diarrhoea (CDAD) usually associated with white cell count up to 15000/μL. In fact, unexplained leukocytosis even in the absence of diarrhoea should prompt clinicians to investigate the patient for CDI. *Clostridium difficile* toxin is positive in 58% of the stool sample of patients with unexplained leukocytosis, compared to 12% in controls (Wanahita, Goldsmith, Marino, & Musher, 2003). Most often in these patients, diarrhoea develops in the next 2 days.

Pseudomembranous colitis is a severe form of CDI. In addition to symptoms of CDAD, sigmoidoscopy in these patients show presence of pseudomembranes, as evidenced by raised white to yellow plaques scattered across colorectal mucosa. Other macroscopic findings that may be seen with or without the presence of pseudomembranes include colorectal wall oedema, erythema, friability, and inflammation. In some cases, pseudomembranes are seen more proximally in the colon via colonoscopy despite being absent at the rectosigmoid area. Hence, the importance of colonoscopy in the early diagnosis of PMC (Seppälä, Hjelt, & Sipponen, 1981).

Ten to twenty-five percent of patients may experience recurrence, which could be due to relapse of the initial infecting strains or due to reinfection with a new strain. Recurrence can occur within days or up to weeks after completion of treatment (Fekety et al., 1997). Often, recurrence represents relapse rather than reinfection. This is proven in a study by Kamboj et al which reported among 102 patients, 85 patients had a second

episode within 8 weeks, 88% of which were relapses. Of 49 second episodes occurring after 8 weeks of initial infection, 65% were relapses (Kamboj et al., 2011). Nevertheless, recurrent diarrhoea in CDI patients may not necessarily indicate recurrence or relapse. Instead, they may have post-infectious irritable bowel syndrome or other inflammatory colitides, concomitant ulcerative colitis or Crohn's disease, or coeliac disease.

Severe CDI may manifest as fulminant colitis. Patients may present with severe lower quadrant or diffuse abdominal pain, diarrhoea and abdominal distension. Fever, hypovolemia, lactic acidosis, hypoalbuminemia, and marked leukocytosis up to 40,000 white blood cells/μL or higher are also common features of fulminant colitis (Bulusu, Narayan, Shetler, & Triadafilopoulos, 2000; Walk et al., 2012; Wanahita et al., 2003). In patients with prolonged ileus, diarrhoea may be less prominent, as a consequence of pooling of secretions in the dilated, atonic colon. Other potential complications of fulminant colitis include toxic megacolon and bowel perforation.

Toxic megacolon is diagnosed clinically. This is based upon the findings of dilated colon up to more than 7cm in its greatest diameter, in association with systemic toxicity. Plain abdominal radiographs demonstrate small intestinal dilation, air fluid levels and scalloping of the bowel wall due to submucosal oedema. On the other hand, patients with perforated bowel manifest as abdominal rigidity, involuntary guarding, diminished bowel sounds, rebound tenderness, and severe localized tenderness in the left or right lower quadrants. Pneumoperitoneum may be seen on plain abdominal radiographs. In both of these conditions, aggressive diagnostic and therapeutic interventions are essential. Prompt surgical referral for evaluation of requirement of colectomy is necessary.

Infrequently, CDI may manifest as protein-losing enteropathy with hypoalbuminaemia (T. J. Lamont, 2016). The consequences include ascites and peripheral oedema, though only few patients may develop these. In CDI, intestinal wall

inflammation permits the leakage of albumin into its lumen and hence colonic loss of albumin. Coupled with inadequate compensatory hepatic albumin synthesis, patients can have hypoalbuminaemia. This condition however, responds well to the treatment of CDI.

As with any infections with enteric pathogens, CDI may complicate the course of inflammatory bowel disease (IBD) (Greenfield et al., 1983; J. T. Lamont & Trnka, 1980). Some institutions report increased incidence of CDAD among patients with IBD (Issa et al., 2007; Rodemann, Dubberke, Reske, Seo, & Stone, 2007). Factors that contribute to this association include frequent antibiotic usage for the treatment of other gastrointestinal pathogens and recurrent hospitalization for management of IBD flares. Rarely, initial bouts of IBD can be triggered by *Clostridium difficile* (Mylonaki, Langmead, Pantes, Johnson, & Rampton, 2004).

The importance of immediate diagnosis and management of CDI in IBD patients lies within the fact that failure to do so will result in patients being inappropriately treated with glucocorticoids and/or immunosuppressive therapy. The challenge to diagnose CDI in IBD patients is that the manifestations are similar – diarrhoea, abdominal pain and low grade fever. Therefore, clinicians should have high index of suspicion of CDI in IBD patients who present with flare of IBD especially those who have recently received antibiotics and/or been hospitalized. In addition, prevalence of *Clostridium difficile* carriage in patients with IBD is high. A study in patients with longstanding IBD showed the frequency of *C. difficile* carriage was higher in IBD patients when compared with healthy volunteers, with 8% and 1% respectively, in absence of recent antibiotics or hospitalization (Clayton et al., 2009).

Similar to adult infection, the prevalence of CDI in paediatric age group is also on the rise. The largest study to date involved 22 hospitals in the United States, by Kim and colleagues revealed increment in annual incidence of CDI among paediatric inpatients, from 2.6 to 4 cases per 1000 admissions per 10000 patient-days, over the 5-year period

to 2006. The median age of children with *C. difficile*-associated disease was 4 years, with 26% being less than 1 year of age and 5% were newborns. The majority of patients had underlying chronic medical conditions (Kim et al., 2008). On the contrary, study by Benson and colleagues observed a significant decrease in the incidence of CDI among inpatients over a 5-year period, which is from 1.024 cases per 1000-patient days to 0.68 cases per 1000-patient days. The same study also reported a concurrent 11% increase in the incidence of community-onset infections. The percentage of patients older than 2 years with CDI however, increased from 46% to 64% for the same period (Benson, Song, Campos, & Singh, 2007).

Benson and colleagues also reported that less than half of CDI is attributed to recent antibiotics exposure (Benson et al., 2007). In Canada, majority of paediatrics patients with CDI (74%) had received antibiotics within preceding 2 months of CDI, with cephalosporins being the most commonly implicated (Morinville & McDonald, 2005). Meanwhile in Turkey, all children with CDAD were on antibiotics therapy, mostly third generation cephalosporins or ampicillin-sulbactam with an aminoglycoside (Oğuz, Uysal, Daşdemir, Oskovi, & Vidinlisan, 2001).

Similar to adult CDI, studies showed prevalence of *C. difficile* is higher in paediatric patients with IBD when compared to those without IBD. Furthermore, prevalence of active disease is greater in *Clostridium difficile* infected patients than uninfected patients. However, in contrast to adult studies, studies in paediatric age group did not find correlation between therapy for IBD with CDI (Enoch, Butler, Pai, Aliyu, & Karas, 2011; Pascarella et al., 2009; Wultańska et al., 2010).

A retrospective study of 68 children with CDI in Italy reported a significant association between the use of proton pump inhibitors and CDI when compared to controls (Turco et al., 2010).

Accurate diagnosis is crucial in the management of CDI. If diagnostic tests are available, diagnostic testing has to be carried out to confirm CDI rather than empirically starting the patient on CDI therapy. All the currently available in vitro assays for *Clostridium difficile* are aids for the clinicians to make an accurate diagnosis. Nevertheless, the diagnosis of CDI remains a challenge to the clinicians and the microbiologists. It is important the diagnostic tests are related to patient's clinical history and physical findings. Rapid and microbiological diagnosis is crucial in CDI.

Specimens that are sent for diagnosis of CDI should be watery or unformed stool. Rectal swabs are not recommended because toxin detections cannot be done reliably. However, in patients with ileus where they may not have diarrhoea, rectal swabs for *Clostridium difficile* may be accepted. Formed stools are not suitable for laboratory testing for CDI in view of approximately 10% of hospitalized patients may be colonized by the bacteria and yet do not have the disease. Usually, single specimen obtained at the onset of symptoms is sufficient to make a diagnosis. In view of the low yield and the possibility of false-positive results, routine testing of multiple stool specimens is not supported as a cost-effective diagnostic practice (Aichinger, Schleck, Harmsen, Nyre, & Patel, 2008). In addition, stool testing for measurement of cure is also not recommended.

Traditionally, cell cytotoxicity neutralization assay (CCNA) had been gold standard for the diagnosis of CDI. This test had excellent sensitivity and specificity. Stool specimen or a *Clostridium difficile* culture isolate is inoculated in a monolayer culture of foreskin fibroblasts. This type of cell is the preferred cell lines because it is the most sensitive for detecting low titre of toxin (1:160 or less) (Tichota-Lee, Jaqua-Stewart, Benfield, Simmons, & Jaqua, 1987). The inoculated cell culture is then observed for cytopathic effect and neutralization with a *C. difficile* toxin B antibody to indicate positivity. However, CCNA is not commonly done in view of high cost and the cell culture is technically demanding.

By far, anaerobic stool culture remains as the most sensitive test for the detection of *C. difficile* (Badger, Ledeboer, Graham, & Edmiston, 2012). This test is not expensive but laborious. The turnaround time for *Clostridium difficile* culture is approximately 2 to 5 days. One of the advantages of this method is that it allows for subsequent toxin type testing for the detection of hypervirulent strains. Anaerobic stool culture combined with CCNA testing has a sensitivity of up to 94% to 100%, and specificity of up to 99% (Badger et al., 2012). The disadvantage however, it is time consuming and therefore limited clinical relevance.

Immuno-enzymatic methods are widely used for laboratory diagnosis for CDI. Toxin enzyme immunoassays (EIAs) are more rapid and easier to perform compared to CCNA. Furthermore, it is not labour intensive and has turnaround time of less than 24 hours. At present, many commercial EIA tests have been introduced to detect toxin A only or detect both toxins A and B. Sensitivity of these tests is 63% to 94%, with specificity of 75% to 100% (Cohen et al., 2010). With this method, sample handling must be done appropriately because failure to do so may result in false negative result due to toxin degradation prior to toxin testing. This test is carried out in more than 90 percent of laboratories because of its commercial availability, rapid turnaround time and cost-effective.

Other method of testing for *Clostridium difficile* is glutamate dehydrogenase (GDH). It is also known as *Clostridium difficile* common antigen. The initial test for GDH is latex agglutination assay. It had a sensitivity of 58% to 68% and a specificity of 94% to 98% (Shanholtzer et al., 1992). Thus, this method is not suitable to be used as routine diagnostic test for CDI in view of its low sensitivity, despite being highly specific, rapid and inexpensive. Furthermore, this method neither offer toxigenic information about the organism, nor yield the isolate itself. For this reason, if it is used as a stand-alone test, this method is insufficient for diagnosis of CDI. The role for GDH testing is more towards a

screening test to rule out negative specimens and to select the specimens for further testing. This is the rationale behind 2-step diagnostic testing for CDI. A study involving 5887 specimens at two different hospitals proved that 2-step testing is cost-effective (Ticehurst et al., 2006). In this study, the GDH test was positive for 16.2% of specimens at one hospital and 24.7% of specimens at the other. Therefore, 75% to 85% of the samples did not require that a cell cytotoxin assay be performed, at a cost savings of between $5,700 and $18,100 per month.

The most up-to-date methods for the detection of *C. difficile* in specimens are the nucleic acid amplification tests. These tests detect either toxin A or B, usually by polymerase chain reaction (PCR). Polymerase chain reaction tests have turnaround times of approximately 30 minutes up to 180 minutes (Carroll, 2011). The specificity is close to 99% and sensitivity of 100% (Badger et al., 2012; Barkin et al., 2012). Polymerase chain reaction detect presence of gene associated with toxigenic organism rather than toxin and clinically significant CDI, and thus will be positive in asymptomatic carriers as well. Positive tests may be confirmed with toxin A/B EIA to determine the clinical relevance. In addition, PCR is more expensive than other test options.

Other diagnostic modalities include endoscopy to visualize pseudomembrane on lower gastrointestinal endoscopy (either sigmoidoscopy or colonoscopy). Histopathological examination is also useful in diagnosis of PMC.

As in any other infection, prevention is always better than cure. *Clostridium difficile* infection can be prevented mainly in two ways (Cohen et al., 2010):

a. Minimize horizontal transmission to reduce exposure to the pathogen.
    i. Hand hygiene
    ii. Contact precaution
    iii. Improvement in hospital layout to reduce transmission
    iv. Identification and treatment of asymptomatic carriers and identification of healthcare workers colonized with *C. difficile*.
b. Practice measures that reduce the risk factors for developing CDI in exposed patients, if exposure has occurred.

CHAPTER 3: MATERIALS AND METHODS/METHODOLOGY

3.1 METHODOLOGY

3.1.1 STUDY DESIGN

Cross sectional study on *Clostridium difficile* detection, the clinical presentation and the associated risk factors for CDI. A study was conducted among inpatients admitted to Hospital Sungai Buloh from 15th June 2015 to 28th February 2016. All unformed stool specimens from patients suspected to have CDI as sent by clinicians were included in the study. The diagnosis of CDI was confirmed when *C. difficile* toxin(s) was(were) detected in the stool specimens.

3.1.2 STUDY DATES/YEAR OF STUDY AND DURATION

Duration of data collection was 8 months (15th June 2015 – 28th February 2016). The total duration of study inclusive of reports writing and submission was 18 months (June 2015 until December 2016).

3.1.3 STUDY SITE AND RATIONALE

This study was carried out in Hospital Sungai Buloh for the reason of it being an infectious disease reference hospital where antibiotics usage is widespread. In addition, the Microbiology laboratory of the hospital is the reference laboratory for identification of anaerobic organisms, one of which is *Clostridium difficile*.

3.1.4 EXPLANATION OF METHODOLOGY IN DETAILS SUCH AS PROCEDURES AND OTHERS

3.1.4.1 COLLECTION OF SAMPLES:

This study involved data collection of the stool specimens routinely sent to Microbiology Laboratory, Hospital Sungai Buloh for isolation and detection of *Clostridium difficile* as per clinical suspicions of CDI by the attending doctors. In this study, duplicate samples from the same patient within a 14-day period were excluded from this study (Peterson & Robicsek, 2009). Samples that were sent from outside Hospital Sungai Buloh were also not be included in this study.

3.1.4.2 IDENTIFICATION OF THE ISOLATES.

Specimens were collected by attending clinicians and were sent to Microbiology Laboratory Hospital Sungai Buloh and were processed according to the Standard Operating Procedure of the laboratory. The isolates were identified based on gram stain, colonial morphology and confirmation of the isolates were done by API 20A (bioMérieux, USA)

Samples were processed within 2 hours of reception. The samples received for detection of Clostridium difficile were tested for presence of Toxin A and Toxin B by a commercial immunochromatographic rapid test kit (Duo Toxin A+B – Check-1 by VEDALAB-France). Results were then entered into the LIS and released for clinician viewing. This commercial rapid test kit is a lateral flow, immunochromatographic rapid test for the qualitative detection of Clostridium difficile toxin A and toxin B in human faeces. Positive tests showed presence of a clearly distinguishable rose-pink coloured band in the positive reaction zone, whereas negative results were evidenced by absence of line in the positive reaction zone.

Subsequently, the samples were cultured on Clostridium-Difficile Agar (CDA) (Thermo). Samples were incubated anaerobically and culture plates were reviewed after 48 hours under the anaerobic chamber. Some of the cultures showed no growth after 48 hours' incubation and these culture plates were incubated for another 48 hours and re-reviewed. The characteristic of *C. difficile* colonies that we looked for was, irregular, greyish colonies with measured approximately 2 to 4mm in diameter. The colonies are non-haemolytic, with presence of characteristic "horse-manure" odour. These colonies were then stained with gram stain, and presence of gram positive or gram variable rods with sub-terminal spores were observed. The presumptive *C. difficile* colonies were then identified by API 20A.

After the isolate was confirmed as *Clostridium difficile*, the antimicrobial susceptibility testing (AST) will be done on the by E-test method (bioMérieux, USA) for the drug of choice for treatment of CDI, specifically vancomycin and metronidazole. The AST was incubated for 24 to 48 hours anaerobically at 37°C. Further incubation may be required if the growth was unsatisfactory. The AST results were interpreted based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). Result was then validated and released out.

3.1.5 FLOWCHART OF STUDY

The summary of the flow of the study were as in the figure below.

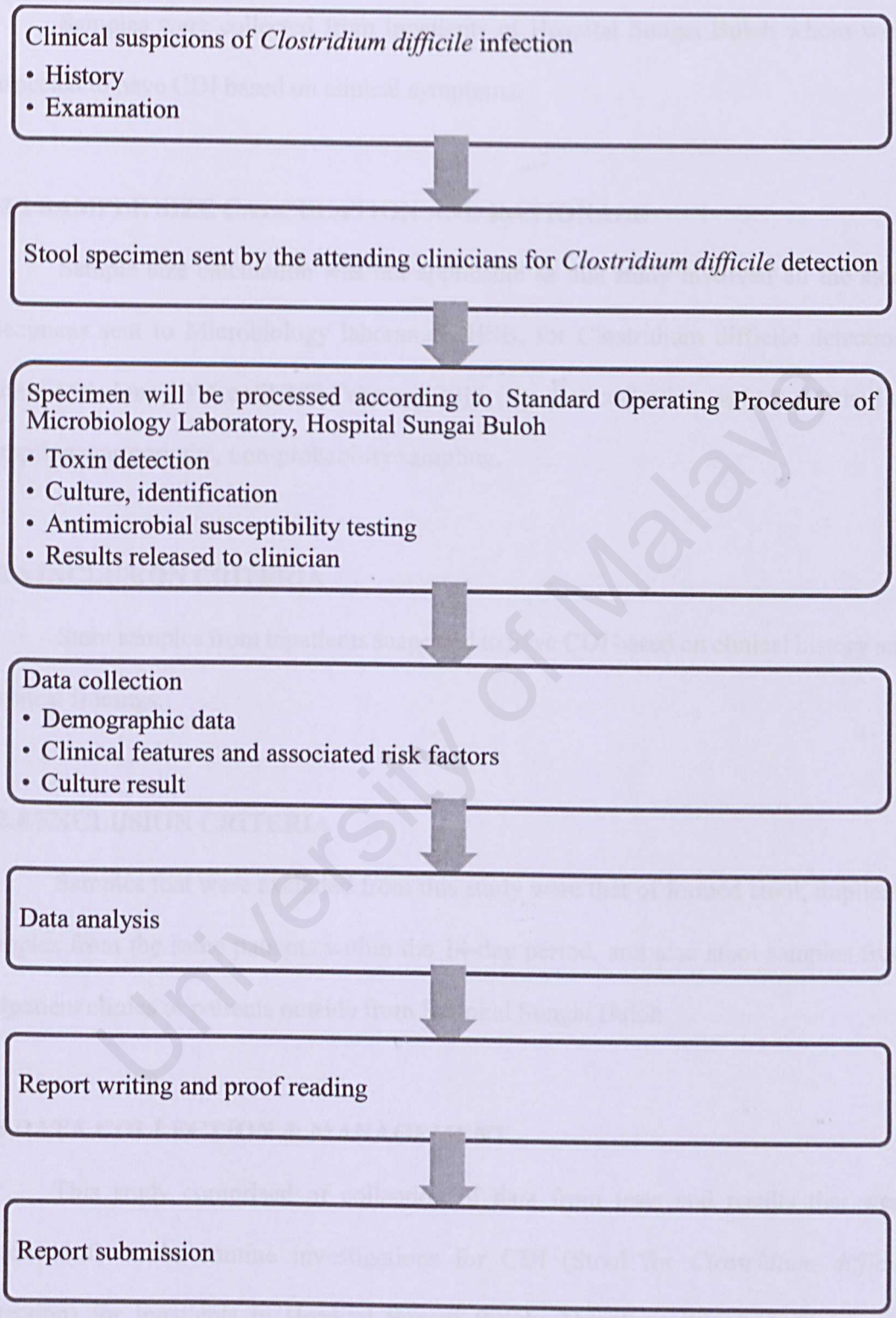

Figure 3.1: Flowchart of the study

3.2 SELECTION AND WITHDRAWAL OF SUBJECT

3.2.1 STUDY POPULATION

Samples were collected from inpatients of Hospital Sungai Buloh whom were suspected to have CDI based on clinical symptoms.

3.2.2 SAMPLE SIZE CALCULATION AND RATIONALE

Sample size calculation was not applicable as this study involved all the stool specimens sent to Microbiology laboratory, HSB, for Clostridium difficile detection, within 15th June 2015 until 28th February 2016 (the data collection period). Method of sampling was periodic, non-probability sampling.

3.2.3 INCLUSION CRITERIA

Stool samples from inpatients suspected to have CDI based on clinical history and physical findings.

3.2.4 EXCLUSION CRITERIA

Samples that were excluded from this study were that of formed stool, duplicate samples from the same patients within the 14-day period, and also stool samples from outpatient clinics or patients outside from Hospital Sungai Buloh

3.3 DATA COLLECTION & MANAGEMENT

This study comprised of collection of data from tests and results that were produced from the routine investigations for CDI (Stool for *Clostridium difficile* detection) for inpatients in Hospital Sungai Buloh. Therefore, this study only used secondary data. Please refer Appendix 1 for data collection form. Missing data and outliers were not be included in the study.

3.4 STATISTICAL CONSIDERATION

Data analysis involved descriptive data. The variables were either numerical or categorical, and would be presented by table. We used Chi-square test and Fischer exact test, which were appropriate. We would find the risk from 2 by 2 categorical variables. These data were then analysed by using Statistical Package for the Social Sciences for Windows (IBM, SPSS version 22.0).

3.5 ETHICS AND PROTECTION OF SUBJECTS

3.5.1 STATEMENT OF ETHICAL ISSUES

This study only involved routine clinical samples that were sent to Microbiology laboratory for inpatients (Hospital Sungai Buloh) suspected to have CDI. The isolation of *Clostridium difficile* from the specimens required up to 5 days or longer for identification and antimicrobial susceptibility testing, and patients might have been discharged before or at the time of results being released. Specific written or verbal consent from patients was not required as the data used were obtained from existing laboratory records and were used anonymously. No additional procedure or intervention was done to the patient.

3.5.2 SUBJECT CONFIDENTIALITY

All the information obtained in this study will be kept and handled confidentially, in accordance with applicable laws and/or regulations. Subject's personal information confidentiality will be maintained. The study protocol, documentation, data, and all other information generated will be held in strict confidentiality. No information concerning the study or the data will be released to any unauthorized third party without prior written approval.

CHAPTER 4: RESULTS

4.1 DEMOGRAPHY OF CASES

Table 4.1: Demographic data of study population (n=198)

Demography	Frequency	Percentage
Gender		
Male	137	69.2
Female	61	30.8
Ethnicity		
Malay	105	53.0
Chinese	39	19.7
Indian	44	22.2
Others	10	5.1
Age group		
Less than 20	11	5.6
20 to 29	28	14.1
30 to 39	57	28.8
40 to 49	40	20.2
50 to 59	33	16.7
60 and above	29	14.6
Discipline/Ward		
Medical	155	78.3
Surgical/Orthopaedics/Neurosurgery	18	9.1
O&G	1	0.5
ICU/HDW	18	9.1
Others	6	3
Length of Stay (days)		
Median	16	
Range (minimum – maximum days)	1 to 99 days	

The demographic data of the sample population is summarized in Table 4.1. The majority of the patients were male, which comprised of 69.2 percent of the study population. Based on this study population, majority of the patients came from the age group 30 years to 39 years old and 40 years to 49 years old with 28.8 percent and 20.2

percent respectively. The majority of samples for *C. difficile* culture came from the medical wards, followed by Intensive Care Units/High Dependency Wards, Surgical/Orthopaedics/Neurosurgery wards and other wards. The patients' duration of admission varied widely, ranging from 1 day to 99 days in the wards. The median length of stay was 16 days.

4.2 PREVALENCE OF CLOSTRIDIUM DIFFICILE INFECTION (CDI)

The data collected over the period of July 2015 to February 2016 were 230 data. However, after duplicate samples were excluded, only 198 data were analyzed.

Out of 198 data analyzed, 12 were positive for *Clostridium difficile* toxin A or toxin B, which resulted in the prevalence of *C. difficile* infection of 6.1 percent amongst inpatients in Hospital Sungai Buloh. Among the 12 isolates positive for *Clostridium difficile* toxin, majority of the isolates were positive for toxin B which accounted for 10 isolates and the remaining 2 isolates were positive for toxin A. In addition, 10 isolates with *C difficile* toxin positive were of male patients, and only 2 isolates belonged to female patients. The association between male gender and risk to develop CDI is not statistically significant, with p-value of 0.350. All of the isolates with positive for *C difficile* toxin were culture negative.

There were 7 cases in which cultures were positive for *Clostridium difficile*, however, the toxins were negative (not detected). These minority of isolates, which covered 3.5 percent of study population may represent *C. difficile* carriers.

4.3 CLINICAL MANIFESTATIONS OF CLOSTRIDIUM DIFFICILE INFECTION

Table 4.2: Clinical presentation of the patients with CDI (n = 12)

Clinical presentation	Frequency (Yes), n (%)	p-value
Fever	5 (41.7%)	1.000
Abdominal pain	5 (45.5%)	0.334
Abdominal distension	0 (0.0%)	1.002
Nausea	6 (54.5%)	0.092
Blood stained diarrhoea	2 (16.7%)	0.181
Leucocytosis	5 (41.7%)	0.544
Hypovolemia	0 (0.0%)	1.000
Dehydration	6 (50.0%)	0.370
Electrolyte imbalances	9 (75.0%)	1.000
Lactic acidosis	0 (0.0%)	0.367

The table 4.2 summarized the clinical presentations of the patients with CDI. The common symptoms were fever, abdominal pain and nausea. Whereas the common clinical signs were electrolyte imbalances, dehydration and leukocytosis. However, based on this study, we cannot prove statistical significance between the clinical signs and symptoms with the diagnosis of CDI (p-value ≥ 0.05).

None of our study population had the clinical manifestations of ileus, perforated bowel, pseudomembranous colitis and toxic megacolon. Hence, these parameters were not analyzed.

4.4 ASSOCIATED RISK FACTORS FOR CLOSTRIDIUM DIFFICILE INFECTION

Table 4.3: The associated risk factors for CDI (n = 12)

Risk factors	Frequency (Yes), n (%)	p-value
Antibiotics		
Imipenem	0 (0.0%)	0.367
Ceftazidime	1 (8.3%)	0.473
Clindamycin	0 (0.0%)	0.610
Fluoroquinolones	2 (16.7%)	0.275
Ampicillin	1 (8.3%)	0.304
Amoxycillin	4 (33.3%)	0.737
Changing of antibiotics	5 (41.7%)	0.566
Intensive Care Unit/High Dependency Ward admission	1 (8.3%)	0.186
Double occupancy room	12 (100%)	0.372
Renal disorders	0 (0.0%)	0.372
Admission to a long-term care facilities within the last year	8 (66.7%)	0.135
Gastric acid reducing therapy	5 (41.7%)	1.000
Hypoalbuminemia	10 (83.3%)	0.347
Retroviral disease	8 (66.7%)	0.248
Autoimmune diseases	0 (0.0%)	1.000
Malignancies	0 (0.0%)	1.000
Cigarette smoking	2 (22.2%)	1.000

The table above summarized the associated risk factors for developing *Clostridium difficile* infection. Out of the 12 patients with CDI, 33.3 percent of them had been exposed to the antibiotic amoxycillin, 2 were exposed to fluoroquinolones, 1 patient

had history of treatment with ampicillin and ceftazidime respectively and none of them were given imipenem and clindamycin. Five out of the twelve patients had changing patterns of antibiotics used in the course of their treatment (p-value = 0.566). All of our patients with CDI are nursed in double or more occupancy rooms (p-value 0.372), however 86.9 percent of our study population were admitted and nursed in similar rooms. Amongst the patients with *C difficile* toxin detected, the large majority of 66.7 percent (8 patients) developed symptoms before admission to hospital, and out these patients, 5 of them had history of admission to hospital within the past one year, and more than half of our CDI patients were retroviral disease patients (66.7 percent respectively). In addition, 83.3 percent of our CDI patients were hypoalbuminaemic (p-value 0.347).

None of our patients had post-pyloric tube feeding, organ transplant, inflammatory bowel disease nor undergoing chemotherapy, and therefore these risk factors were not analyzed.

4.5 MICROBIOLOGY RESULTS

As aforementioned, out of 198 data analysed, only 12 isolates were positive for *Clostridium difficile* toxin, which gave the prevalence of CDI among our study population of 6.1 percent. In addition, culture positive and toxin negative isolates were 3.5 percent.

The table 4.4 showed the range of duration taken between sample collection to the sample receipt by the laboratory. From this table, we can conclude that majority of the specimens were received within 4 hours after collection. Majority of the stool samples received were of soft stool consistency (79.3 percent), however were not watery. Only 1 out of 198 stool specimens analysed was blood-stained.

Table 4.4: Duration between sample collection to specimen receipt by the laboratory (n=198)

Duration	Frequency (n)	Percentage
Less than 1 hour	26	13.1
1 hour to 1 hour 59 minutes	53	26.8
2 hours to 3 hours 59 minutes	43	21.7
4 hours to 5 hours 59 minutes	24	12.1
More than 6 hours	52	26.3

Out of the 12 isolates with *C. difficile* toxin positive, only 3 samples were watery stool, giving percentage and p-value of 25 percent and 0.716 respectively, the remaining specimens were semisolid/soft in consistency. In addition, 8 out of the 12 isolates positive for the toxin were sent to the laboratory within 4 hours of collection, and only 3 (25 percent) were sent within 1 hour. All the culture for *C difficile* were negative for all these 12 isolates. From our study, majority of our patient with CDI were treated with metronidazole alone (n = 8, 66.7 percent).

In the contrary with toxin positive isolates (cultures were negative), 6 out of the 7 isolates with *Clostridium difficile* isolated from culture were received by the laboratory more than 4 hours after specimens were collected, and only 1 was received in less than 1 hour after specimen collection. All the *Clostridium difficile* isolated were sensitive to both antimicrobials used to treat CDI, specifically metronidazole and vancomycin.

We also look at other microbiological investigations sent for these patients and we found either the investigations were negative (1 isolates) or not sent (6 isolates).

CHAPTER 5: DISCUSSION

In our study, majority of the patients were male, which comprised of 69.2 percent of the study population. This corresponded to the national statistic that showed slight majority of Malaysian population were male, which comprised of 51.7 percent (16.4 million) of the total population in Malaysia (Malaysia, Jabatan Statistik, 2016). A clinico-epidemiological study done Kuala Lumpur also revealed that the majority of patients with CDI were male, with male to female ratio were 3.5:1 (NA & TZAR, 2013). However, another prevalence study done in Kelantan, Malaysia revealed that majority of the patients with CDI are female (54.2 percent) (Hassan et al., 2012).

If we look into distribution of study population based on ethnicity, the demography of the study population again matched the national ethnic composition in which Malays constitute the vast majority of the population. The same pattern were seen in study by Hassan et al which showed Malay patients were the predominant ethnic group (Hassan et al., 2012). Interestingly, study done in HUKM (NA & TZAR, 2013) were different in the sense that majority of their CDI patient were Chinese, followed by Malays which accounted for 55.6 percent and 44.4 percent respectively. These patterns could be due to distribution of ethnic groups based on geographical area.

From this study, approximately half of the patients were aged 30 to 49 years old. There were low number of paediatrics patients included in the study. The low numbers of patients from the younger age group could be due to either paediatrics patient were less likely to have CDI or the clinical awareness amongst the clinicians were low. However, various studies have shown that CDI do occur in children (Benson et al., 2007; Kim et al., 2008).

The majority of samples for *C. difficile* culture came from the medical wards, followed by Intensive Care Units/High Dependency Wards, Surgical/Orthopaedics/Neurosurgery wards and other wards. Again, this findings corresponded to the previous two prevalence study done in Malaysia (Hassan et al., 2012; NA & TZAR, 2013). The patients' duration if admission varied widely, ranging from 1 day to 99 days in the wards. The median length of stay was 16 days.

This study showed the prevalence of CDI in Hospital Sungai Buloh was 6.1 percent which is similar to study by NA and TZAR. When compared to study by Hassan et al, which revealed that the prevalence of CDI in their centre was 13.7 percent. Their prevalence was higher possibly because the authors had included loose stool samples sent for various clinical indication. In contrast, our study had included only stool samples sent for patients with suspected CDI. In addition, lack of suspicion and awareness of CDI could be a contributing factor for our prevalence of CDI. Further studies have to be conducted to ascertain this theory. None of our toxin-positive isolates grew on culture, despite stool culture being the most sensitive test to detect *C. difficile* (Badger et al., 2012; Cohen et al., 2010). Even though culture has slow turn-around time, it remains the standard. Culture is an important tool to aid diagnosis in patients with high level of suspicion for CDI despite negative GDH or toxin detection. In addition, culture can be used in the evaluation of new diagnostic tests for toxigenic *C. difficile* and also for strain typing and susceptibility testing (Reller et al., 2007). From our study, the percentage of *Clostridium difficile* carrier is 3.5 percent, whereas according to American College of Gastroenterology, percentage of carrier among healthy adult ranged from 5 to 15 percent, and up to 84.4 percent in new-born and healthy infants and in patients in long term health care facilities, the rate could be up to 57 percent (Surawicz et al., 2013).

The primary symptom of CDI is watery diarrhoea. These episodes of diarrhoea could be accompanied by lower abdominal pain and cramping, elevated white cell count and fever (T. J. Lamont, 2016). In our study, these clinical presentations were also the commonest signs and symptoms recorded. Our patients mostly have fever and abdominal pain, on top of the diarrhoea. Nausea and leukocytosis also were frequently seen in our patients. However, we must not forget that other causes of diarrhoea could also present with similar signs and symptoms. Therefore, early diagnosis of CDI can be challenging. As CDI is commonly associated with previous exposure to antibiotics, history of prior antibiotics usage should raise the suspicion of CDI. Patients can present with symptoms after several days and up to months after exposure to antibiotics. Other clinical manifestations of CDI include other nonspecific signs and symptoms such as nausea, vomiting, lethargy, fever, dehydration, and tachycardia (Stanley et al., 2013). Again, dehydration and electrolyte imbalances associated with dehydration were also frequent in our patients. Shock and ileus indicate severe and complicated CDI (Stanley et al., 2013; Surawicz et al., 2013). However, none of our patients had signs and symptoms of severe CDI. Despite our patients' clinical presentation of CDI were almost similar to other studies, we were unable to find statistically significant correlation between the clinical presentations and CDI among our patient.

Clostridium difficile infection is usually associated with prolonged course of antibiotics. Usage of antibiotics less than 3 days was associated with lower risk of developing CDAD (Wiström et al., 2001). Another study in Thailand showed that prevalence of CDAD are higher in the antimicrobial-treated group when compared to controls, with percentage of 14.3 percent to 0.7 percent respectively (Putsathit, Kiratisin, Ngamwongsatit, & Riley, 2015). As aforementioned, the aantibiotics that were linked to CDI include aminoglycosides, penicillins, cephalosporins, second- and third-generation beta lactam compounds, clindamycin, erythromycin, lincomycin, metronidazole,

rifampin, trimethoprim-sulfamethoxazole, amphotericin B and the fluoroquinolones (Gerding, 2004; Gerding et al., 1995). From our study, the antibiotic most commonly associated with CDI was amoxycillin which was 33.3 percent from the CDI patients (p-value = 0.737). In addition, nearly half of the patients with CDI had changing patterns of antimicrobial therapy during their admissions to ward. However, the reasons for changing the antibiotics and the total durations of antibiotic therapy were not recorded in our study.

It is a well-known fact that CDI can spread via contaminated hands of healthcare workers and patients, and also contaminated fomites and equipment. Therefore, being nursed in a double or more occupancy rooms served as an important route of spread of CDI, particularly when infection control measures were breached. All of our patients with CDI were not nursed in isolation room, however, they were put on contact precaution. It is quite tricky to attribute the CDI among these patients were due to nosocomial spread or vice-versa because the large majority of our patients with CDI developed symptoms before their admission to hospital. However, it is important to consider that 66.7 percent of the patients with CDI had history of admission to hospital within the past one year. Further studies need to be conducted to look at prevalence of community-acquired CDI among our population.

Immunocompromised states such as HIV are exposed to CDI. These group of patients require frequent visits to medical care and hence hospital admission and exposure to antibiotic therapy (Raines & Lopez, 2011). Therefore, retroviral disease is one of the risk factors for CDI (Bignardi, 1998; Stanley et al., 2013). This corresponded to our findings which showed that out of 12 patients with CDI, 66.7 percent of them were HIV positive patients. In contrast, a study done in France stated that HIV infection alone was not associated with CDI (Hutin et al., 1997). The comorbidities associated with HIV infection also involved exposure to many of the risk factors for CDI (Collini, Bauer, Kuijper, & Dockrell, 2012).

Hypoalbuminaemia is one of the indicator of severe CDI (Surawicz et al., 2013). It associated with protein loss from albumin leakage and in severe cases, patients may present with anasarca (Bartlett & Gerding, 2008). From our study, 88.3 percent of our CDI patients were hypoalbuminaemic. Nevertheless, the association to CDI was not statistically significant (p-value 0.347). In addition, in this study, there were no cases of severe CDI isolated. When compared to the control group, in actual fact, majority of our patients were hypoalbuminaemic as evidenced by 90.3 percent of those without CDI had low serum albumin levels.

Ideally, stool sample for *C. difficile* culture should reach the laboratory for processing within 1 hour of collection if transported at room temperature, and if delay is unavoidable, sample should either be transported within 24 hours at 4 degree Celsius, or at -20 degree Celsius (more than 24 hours) (Miller, 1999). Most of our samples reached the laboratory within 24 hours after collection. However, only 13.1 percent of the sample were sent to the laboratory within the recommended time. Among 26 samples that reached the laboratory within 1 hour, only 3 were positive for *C. difficile* toxin. Nevertheless, majority of the stool sample positive for toxin were sent to the laboratory within 4 hours. From our study, all the samples that were positive for *C. difficile* toxin were negative for culture. Interestingly, there were 7 isolates that were tested negative for *C. difficile* toxin but were positive for culture. Out of these culture positive samples, only 1 was sent to the laboratory within the recommended 1 hour transportation time. Further studies need to be conducted to study the correlation between specimen transportation time to the laboratory and the isolation of *C. difficile* from culture.

According to American College of Gastroenterology, mild to moderate CDI can be treated with oral metronidazole 500mg thrice a day for 10 days, whereas severe CDI should be treated with vancomycin 125mg 4 times a day for 10 days (Surawicz et al., 2013). Our patients were mostly treated with metronidazole alone. Other than treatment

with antimicrobial, the predisposing risk factor(s) for CDI should be removed or discontinued whenever possible.

This study is not complete without its limitations. First and foremost, the study sample was very small and the duration of study was brief. Therefore, this could have affected our results and statistical associations. In addition, as our study involved the collection of secondary data, we relied heavily on documentation of clinical information in the patients' clinical notes. Some of the information we were looking for were not documented. Lastly, in our study, we did not differentiate between community acquired CDI and hospital acquired.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

1. The prevalence of CDI in Hospital Sungai Buloh is relatively low (6.1%) and the cases were mild to moderate in severity.
2. The most common symptoms other than diarrhoea were fever, abdominal pain and nausea, whereas the common signs were dehydration, electrolytes imbalances and leucocytosis.
3. The most common predisposing antibiotic in our patients with CDI was amoxycillin
4. All our patients were nursed in double or more occupancy room. Eight (66.7%) had history of admission to long-term healthcare facilities within the past 1 year and 10 (83.3%) of the CDI patients were hypoalbuminaemic.
5. There were no statistically significant association between the risk factors and CDI in our patients.
6. Our study provides a baseline prevalence of CDI among patients in Hospital Sungai Buloh.

6.2 RECOMMENDATION

1. It is essential to increase the awareness regarding CDI among our clinicians and also microbiologists. In addition, good communication, sharing of data and proper documentation of clinical information are also important.
2. Further studies may need larger sample size and longer duration of study so that more concrete statistical association can be determined. In addition, study of community acquired CDI and hospital acquired CDI should also be carried out as more data are suggesting of occurrence of community acquired CDI.

3. Continuous surveillance and collection of data are crucial in order to assess severity of CDI among our patients, and also to look at pattern of infection.
4. Judicious use of antibiotics and also regular audit by antibiotic stewardship team may help to reduce the risk of developing CDI and hence reducing mortality and morbidity associated with CDI.
5. Adherence to infection control measures, especially hand hygiene, are one of the most important preventive methods for CDI. Regular education and audit among healthcare personnel on compliance on hand hygiene should be carried out.

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APPENDIX

Appendix 1

DATA COLLECTION FORM

Title: Prevalence of *Clostridium difficile* Infection in Hospital Sungai Buloh and The Associated Risk Factors

Data number: CD _____

Section 1: Demographic data		
	Code	
Patient ID	ID	CD _____
Date of Admission	DOA	
Date of Discharge	DOD	
Admitting ward/Discipline	Ward	
Age (in years)	Age	
Gender	Gender	☐ Male ☐ Female
Race	Race	☐ Malay ☐ Indian ☐ Chinese ☐ Other (please specify)
Section 2: Clinical history		
Diagnosis	DX	
Date of onset of symptoms	DOS	
Clinical presentation	SX	☐ Fever, temperature: ……. ☐ Abdominal pain. Yes / No ☐ Abdominal distension. Yes / No ☐ Nausea. Yes / No ☐ Diarrhoea. Blood-stained: Yes / No ☐ White cell count (specify) …… ☐ Hypovolemia. Yes / No ☐ Dehydration. Yes / No ☐ Electrolyte imbalance. Yes / No ☐ Lactic acidosis. Yes / No ☐ Albumin level (specify) …….. ☐ Ileus. Yes / No ☐ Perforated bowel. Yes / No ☐ Pseudomembranous colitis. Yes / No ☐ Toxic megacolon. Yes / No

Section 3: Associated risk factors		
Risk factors	RF	☐ Underlying medical illness (specify): …….. ☐ Antibiotics o Imipenem. Yes / No o Ceftazidime. Yes / No o Clindamycin. Yes / No o Fluoroquinolones. Yes / No o Ampicillin. Yes / No o Amoxicillin. Yes / No ☐ Changing patterns of antibiotics used. Yes / No ☐ Intensive care unit admission. Yes / No ☐ ≥ Double occupancy room. Yes / No ☐ Renal disorders. Yes / No ☐ Post-pyloric tube feeding. Yes / No ☐ Admission to a long-term care facilities within the last year. Yes / No ☐ Gastric acid reducing therapy/Proton pump inhibitor. Yes / No ☐ Hypoalbuminaemia. Yes / No ☐ Human Immunodeficiency Virus. Yes / No ☐ Organ transplant. Yes / No ☐ Autoimmune disease. Yes / No ☐ Chemotherapy. Yes / No ☐ Inflammatory bowel disease. Yes / No ☐ Malignancy. Yes / No ☐ Smoking. Yes / No

Section 4: Result		
Time/date specimen collected	DOC	
Time/date specimen received	DOR	
Macroscopic	MACRO	☐ Watery. Yes / No ☐ Blood-stained ☐ Not blood-stained
Toxin detection	TOXIN	☐ Toxin A detected ☐ Toxin B detected ☐ Not detected
Culture result	CULTURE	☐ *Clostridium difficile* isolated ☐ *Clostridium difficile* not isolated
VITEK ANC/API 20A result	ISOLATE	Specify:
Antimicrobial susceptibility test	AST	☐ Metronidazole ☐ Vancomycin

PREVALENCE OF CLOSTRIDIUM DIFFICILE INFECTION IN HOSPITAL SUNGAI BULOH AND THE ASSOCIATED RISK FACTORS

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