PREVALENCE AND ASSOCIATED RISK FACTORS OF *CLOSTRIDIUM DIFFICILE* INFECTION IN TYPE 2 DIABETES MELLITUS PATIENTS TREATED WITH ANTIBIOTICS

BY

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ABBREVIATION

CD	*Clostridium difficile*
CDAD	*Clostridium difficile* associated disease/diarrhea
CDI	*Clostridium difficile* infection
CKD	Chronic kidney disease
EIA	Enzyme immunoassay
GDH	Glutamate dehydrogenase
HbA1c	Glycosylated haemoglobin
IBD	Inflammatory bowel disease
PCR	Polymerase chain reaction
PPI	Proton pump inhibitor
USM	University Sains Malaysia

ABSTRAK

PREVALEN JANGKITAN *CLOSTRIDIUM DIFFICILE* DAN FAKTOR-FAKTOR YANG BERKAITAN DI KALANGAN PESAKIT TYPE 2 DIABETES MELLITUS YANG MENERIMA ANTIBIOTIK

Latar belakang

Salah satu jangkitan kuman di hospital yang paling kerap berlaku adalah jangkitan kuman yang disebabkan oleh kuman *Clostriduim difficile.* Jangkitan kuman ini juga merupakan antara penyebab cirit- birit di kalangan pesakit yang mengambil antibiotik. Jangkitan kuman ini sedang meningkat naik dan ia berkait rapat dengan risiko kematian yang tinggi dan morbiditi yang tinggi di kalangan pesakit di hospital. Ada yang menganggap diabetes mellitus sebagai salah satu faktor risiko untuk mendapat jangkitan kuman ini dan najis daripada pesakit yang menghidap diabetes mellitus mempunyai jumlah toxin *Clostridium difficile* yang tinggi berbanding orang biasa.

Metodologi

Kajian ini adalah satu kajian hirisan lintang yang dijalankan daripada bulan Ogos 2009 sehingga Mei 2010 di Hospital Universiti Sains Malaysia. Pesakit di wad perubatan yang berumur 30 tahun dan ke atas, yang menghidap type 2 diabetes mellitus dan menerima rawatan antibiotik, sekiranya bersetuju untuk mengambil bahagian dan memberi keizinan akan dimasukkan di dalam kajian ini. Jumlah pesakit yang diperlukan adalah seramai 159 pesakit. Spesimen najis akan dikumpul dan dihantar ke makmal

untuk dikaji bagi mengesan toxin. Sampel darah juga akan diambil daripada pesakit. Maklumat yang diambil adalah data demograpfi, rawatan antibiotik, penemuan klinikal dan hasil keputusan makmal dan kesemua data ini akan direkodkan di dalam borang pengumpulan data yang standard. Data-data ini akan dianalisa untuk mencari kajian data yang ada kaitan dengan jangkitan kuman *Clostridium difficile*.

Keputusan

159 pesakit telah terlibat di dalam kajian ini dan purata umur mereka adalah 60.47±10.98. Daripada jumlah ini hanya 14 (8.8%) sampel najis yang memberi keputusan positif bagi toxin *Clostridium difficile*. Purata umur bagi pesakit yang positif untuk jangkitan ini adalah 53.79±14.19 dan purata umur bagi pesakit yang tidak mempunyai jangkitan ini adalah 61.11±10.46. Kami mendapati tiada keputusan makmal yang berkait dengan jangkitan ini dan ini termasuk "total white count", urea, albumin, creatinine dan HbA1c.

Keputusan analisis kajian ini menunjukkan pesakit yang menghidap jangkitan kuman dalam darah berkait rapat dengan CDI (p=0.043) dan antara symptom yang berkait rapat dengan CDI adalah cirit-birit (p=0.002) dan sakit perut (p=0.001). Imipenem (p=0.006, OR; 30.1), cefoperazone (p=0.036, OR;9.5) dan proton pump inhibitor (p=0.041, OR;13.7) pula mempunyai kaitan yang signifikan dengan jangkitan *Clostridium difficile*

Rumusan

Prevalen jangkitan *Clostridium difficile* bagi pesakit type 2 diabetes mellitus yang menerima antibiotik adalah 8.8% dan antara antibiotik yang mempunyai kaitan signifikan dengan jangkitan ini adalah imipenem dan cefoperazone. Penggunaan proton pump inhibitor juga mempunyai kaitan signifikan dengan jangkitan ini. Dengan penemuan ini, ia dapat membantu mengenalpasti pesakit yang berisiko tinggi untuk mendapat jangkitan *Clostridium difficile.*

ABSTRACT

PREVALENCE AND ASSOCIATED RISK FACTORS OF *CLOSTRIDIUM DIFFICILE* INFECTION IN TYPE 2 DIABETES MELLITUS PATIENTS TREATED WITH ANTIBIOTICS

Background

Clostridium difficile infection (CDI) is one of the most common nosocomial infection and it is one of the leading cause for antibiotic associated diarrhea. CDI is currently on the rise and it is associated with high morbidity and mortality among in hospital patients. Some authors consider diabetes mellitus as a risk factor for CDI and stools from diabetic patients have revealed a higher concentration of *Clostridium difficile* toxin compared to healthy individual.

Methodology

This study was a cross-sectional study and was performed from August 2009 until May 2010 in Hospital University Science Malaysia (HUSM). All adults aged 30 years and above with documented history of type 2 diabetes mellitus who were admitted to general medical wards and received antibiotics, willing to participate and consented were enrolled in this study. A total of 159 patients were involved in this study. Stool specimens were collected and tested for presence of toxin and few blood investigations were taken. Demographic data together with laboratory results were collected and recorded in a standard data collection sheet. Variables were analyzed

and chi square test and logistic regression test were used to identify significant association with CDI.

Result

Among 159 patients enrolled in this study, 14 of 159 (8.8%) were tested positive for the presence of *C. difficile* toxins. The mean age of the patients involved was 60.47 ±10.98. Those in the positive *C. difficile* group has a mean age of 53.79 ±14.19 and those in the negative *C. difficile* group has a mean age of 61.11 ±10.46. We found that none of the laboratory markers were significantly associated with CDI. These included total white count, albumin, urea, creatinine and HbA1c. From univariate analysis, bacteremia was associated with CDI (p=0.043) and among the symptoms, diarrhea and abdominal pain were associated with CDI with p=0.002 and p=0.001 respectively. From multivariate analysis we identified that imipenem (p=0.006, OR; 30.1), cefoperazone (p=0.036, OR; 9.5) and proton pump inhibitor (p=0.041, OR; 13.7) were strongly associated with CDI.

Conclusion

The prevalence of *Clostridium difficile* infection in patients with type 2 diabetes mellitus who were treated with antibiotics was 8.8% and among the antibiotics strongly associated with CDI were imipenem and cefoperazone. Proton pump inhibitor was also strongly associated with CDI and presence of bacteremia was also associated

with CDI. These findings will allow clinician to identify patients with high risks of developing CDI.

1. INTRODUCTION

1.1 STUDY BACKGROUND AND RATIONALE

Nosocomial infections are widespread and they are important contributors to morbidity and mortality. *Clostridium difficile (C. difficile)* is one of the important nosocomial micro-organism which commonly infected hospitalized patients. This infection can be devastating to some and fatal to others.

The epidemiology of *C. difficile* infection has changed remarkably over the last few years both globally and in Asia due to the increasing awareness of this infection. Few outbreaks that had occurred , revealed a new strain of *C. difficile* as the cause for the outbreak (Luke *et al*, 2008). This new strain of *C. difficile* which is called NAP1/BI/027 is responsible for the more severe form of infection and can be difficult to treat (Luke *et al,* 2008). In view of the changing epidemiology, the restrictive term of *Clostridium difficile* – associated diarrhea (CDAD) has been changed to *Clostridium difficile* infection (CDI), which indicates a wider spectrum of clinical disease that is not limited to diarrhea only (Luke *et al*, 2008). These terminologies however are interchangeable. The infection unfortunately has been on the rise and causes an impact particularly on the financial department in the health system. In North America the incidence has been increasing over the past 5 years and it has been estimated that the US healthcare system spent about $ 1.1 billion each year on management of this infection (Kuijper *et al,* 2006). Data from United Kingdom's health Protection Agency also showed that the incidence of CDI has increased since 1990. Unfortunately data on the incidence of CDI

in Asia are more limited but the available data suggests that the infection did not spare Asia (Luke *et al*, 2008). There is an obvious increasing trend seen in countries including Singapore, Japan, Korea, India and Taiwan (Luke *et al*, 2008). Investigators from Asia and Middle East reported a higher prevalence of toxin A negative, toxin B positive strains which are associated with a more severe disease outcome (Koh *et al*,2007).

Most cases of *C. difficile* have occurred in patients with traditional risks factors for antibiotic-associated colitis but an increasing proportion of patients with this infection do not have these risk factors and even a previously healthy people living in the community can develop this infection (Khanna *et al*, 2009). The increasing incidence of CDI has been attributed to the growing use of antibiotics and the more virulent antibiotic-resistant strain *C. difficile* (Luke *et al*, 2008). A study had shown an association between the infection of hypervirulent strain and the use of fluoroquinolones for community acquired pneumonia (Polygreen *et al*, 2007). Diabetes mellitus has also been associated as a risk factor for CDI as it is known to cause immune system suppression hence increases the susceptibility to infection. Unfortunately there are limited studies done on CDI in patients with diabetes mellitus.

This study is designed to determine the prevalence of CDI in hospitalized patients with diabetes mellitus who were exposed to antibiotics. We hypothesized that CDI in this group of patients will be higher than the general population. We are also looking at the risk factors which contribute to the infection in this group of patients. In addition we

are also looking at the clinical features and which antibiotics would be associated with the infection. We hope that the data collected in this study would provide additional knowledge about the infection and help in identifying potential patients that may develop the CDI. We also hope that with this study, doctors and health-care workers will be more aware about CDI and will include it as one of the differential diagnosis in treating nosocomial infection especially among diabetic patients.

1.2 HISTORY OF *CLOSTRIDIUM DIFFICILE*

C. difficile was first discovered in 1935 by Hall and O'Toole from the meconium and faeces of newborn infants. It was then referred to as *Bacillus difficilis.* It was classified as commensal since it was found in stools of healthy infants.

In 1977, Larson *et al* showed that stool filtrates from a patient with pseudomembranous colitis had a cytotoxic effect on tissue culture cells, suggesting the presence of a toxin. At the same time, investigators in the United States showed that clindamycin and other antibiotics induced a lethal caecitis in hamsters (Barlett *et al*, 1977). An organism identified as *C. difficile* was isolated from the animals and was shown to be the source of the toxin.

After the detection of *C. difficile* and its toxin from patients with pseudomembranous colitis, *C. difficile* has since become established as a major cause of nosocomial diarrheal infection. Recently it has caused some major outbreak in Europe, Canada and the United States.

1.3 OVERVIEW OF *CLOSTRIDIUM DIFFICILE* INFECTION

1.3.1 DEFINITION

Clostridium difficile infection (CDI) is defined when there is at least one episode of diarrhea which is an unformed stool that conforms to the shape of a specimen collection container. The stool must be tested positive for the presence of *C. difficile* toxin (*Clostridium difficile* Policy 2010).

Other clinical pictures that are compatible with CDI are ileus and toxic megacolon. Ileus in the context of CDI is defined as signs of severely disturbed bowel passage such as vomiting and absence of stool, combined with radiological signs of bowel distension and finally toxic megacolon is defined as a radiological signs of distension of the colon combined with signs of severe systemic inflammatory response (Bauer *et al,* 2009).

1.3.2 PATHOGENESIS

C. difficile is a gram positive anaerobic bacilli and it is transmitted primarily from human (either colonized or infected patients), though any devices or surfaces contaminated with infected feces may serve as reservoirs for *C. difficult* spores. It is transmitted via fecal-oral route. These spores are resistant to exposure of air, heat and drying and can survive in the environment for months. The spores are the culprit for transmission of infection.

Based on hamster models, most ingested vegetative cells are killed in the stomach, with only 1% of the inoculum passing into the small bowel. However *C. difficile* spores are acid resistance and readily passed through the stomach. They may germinate in the small bowel upon exposure to bile acids (Susan M P, 2004). A number of virulence factors like flagellae and hydrolytic enzymes produced by the organism have been associated with the development of disease (Susan M P, 2004).

There are two predisposing factors that must be present for developing *C difficile* infection, which are disruption of the normal gastrointestinal flora and acquisition of the organism from an exogenous source(I Tonna, 2005). Other factors involved in the development of the infection include host susceptibility, virulence of the *C. difficile* strain concerned and the nature and extent of antimicrobial exposure (I Tonna, 2005).

There are more than 400 strains of *C difficile* and only toxin producing strains produce disease (I Tonna, 2005). *C. difficile* produces at least two distinct toxins which had been labeled as toxin A and toxin B. Toxin A and B have been shown by nucleotide sequencing to be located in close proximity to each other, encoded by two separate genes *(tcdA* and *tcdB)* on the same chromosome (Vaishnavi, 2010). Previous animal studies have suggested that only toxin A mediates diarrhea and enterocolitis but when toxin A negative/toxin B positive strains of *C. difficile* are isolated from patients with antibiotic associated diarrhea and colitis, this indicated that toxin B may also be pathogenic in humans (Perry H, 2009).

Biochemical and molecular studies have shown that the main clinical symptoms and signs of CDI can be largely explained by the action of these two toxins (Rupnik M et al, 2009). Toxin A is a lethal enterotoxin and minute quantities can stimulate fluid secretion in intestine and it causes extensive damage to the epithelial lining of the intestine by disrupting the epithelium villus followed by damage to the brush border membrane (Vaishnavi, 2010). This is accompanied by extensive neutrophil infiltration resulting in massive inflammation. Toxin A is also a cytotoxin which disrupt the tight junctions of the intestinal epithelium. Toxin B is also a cytotoxin and the cytotoxic activity is similar to that of toxin A but it is more potent than toxin A.

The toxins get transported into the cytoplasm where they act on small guanosine triphosphate binding proteins known as Rho proteins which are associated with actin

polymerization, maintenance of cytoskeletal architecture and the cell movement. A severe inflammatory reaction in the lamina propria with the formation of micro-ulcerations of the colonic mucosa that is covered by a pseudomembrane occurs due to the activity of the toxins (Vaishnavi, 2010). Besides toxin A and B, another toxin which is called a binary toxin has also been identified. This was first reported in 1988 but not considered important until when binary toxin producers make up the majority of the *C. difficile* strains isolated in the large outbreaks of the disease in Canada and United States (Thomas V R, 2006). A correlation between binary toxin production and severity of diarrhoea has been demonstrated and more community-acquired *C. difficile*-associated diarrhoea was found to be caused by binary toxin producers (Thomas V R, 2006).

C. difficile has been found in approximately 3% of normal adults and up to 40% of hospitalized patients (Bartlett JG *et al*, 2004). Lawrence (2007) has claimed that about 20% of hospitalized adults are *C. difficile* carriers and in long term care facilities the carriage rate may approach 50% (Riggs M, 2007). Although asymptomatic, they shed pathogenic organisms and serve as a reservoir for environmental contamination. Patients who have *C. difficile* as an asymptomatic organism in their intestine on hospital admission, will only develop the disease after they are treated with antibiotics. Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonization but if the normal colonic flora is altered, resistance to colonization is lost. Therefore any factors associated with alteration of the normal colonic flora increases the risk of colonization by *C. difficile*.

The extent of clinical manifestations depends on the immune response to *C difficile* and patients with low anti-toxin A IgG levels manifest more severe disease unlike those with higher levels who usually recover spontaneously (I Tonna, 2005). A study by Kyne *et al*,1999 showed that patients who were recently colonized with *C. difficile* and had a high serum antibody response to *C. difficile* toxin A were usually protected against diarrhea and became asymptomatic carriers. In contrast, patients who had low serum antibody response to toxin A had a much greater risk of diarrhea and these findings suggest that antibody response to toxin A protects against the development of *C. difficile* diarrhea (Susan M P, 2004). The risk of diarrhea is also related to the virulence of the infecting *C. difficile* strain.

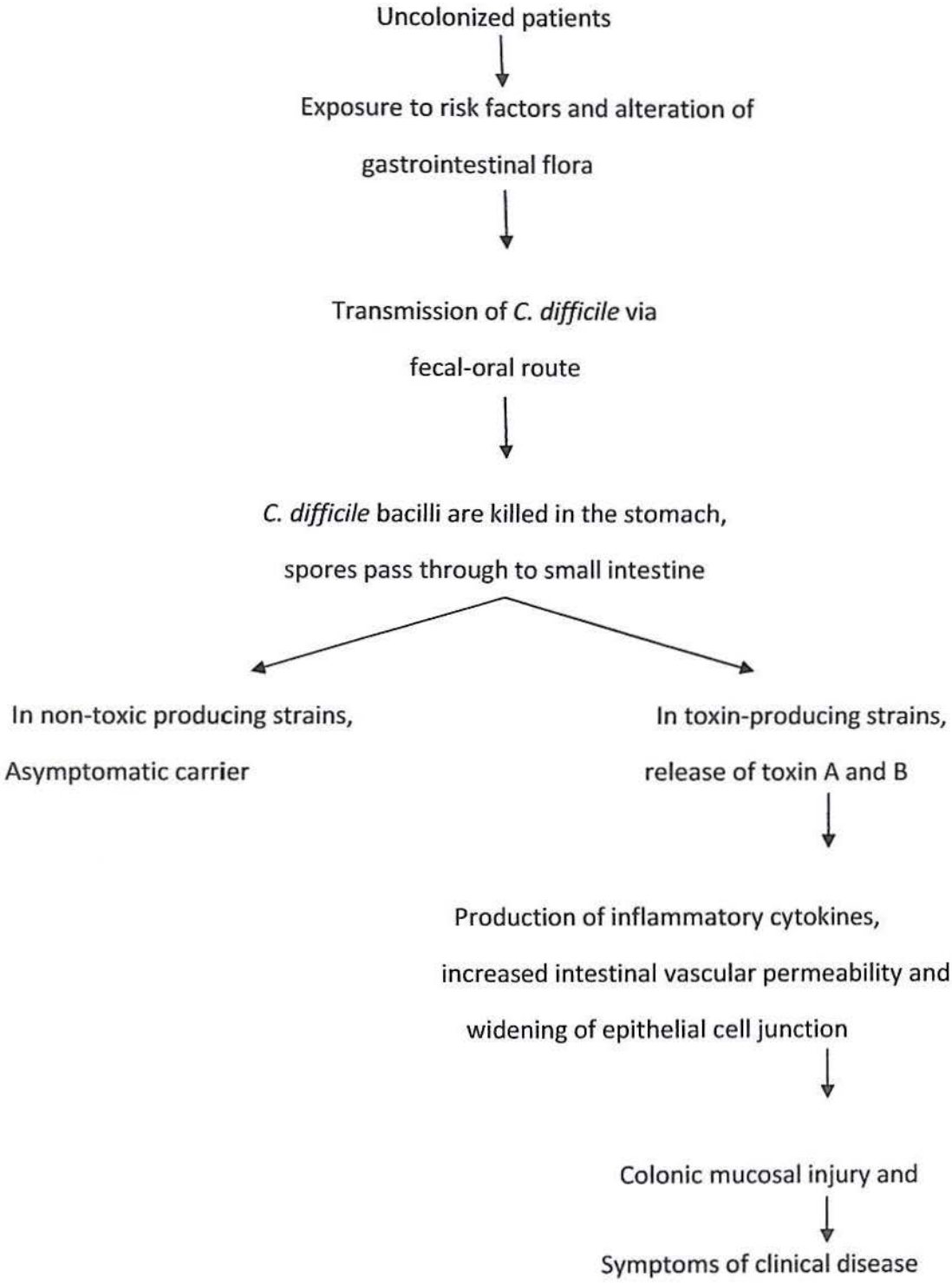

Figure 1. Pathogenesis of *C. difficile* disease (adapted from April D. Miller, PharmD et al, P&T, 2006:510-520)

1.3.3 RISK FACTORS FOR *CLOSTRIDIUM DIFFICILE* INFECTION

There are many risk factors that have been proposed to increase the risk of acquiring *C. difficile* infection.

(a) Drugs

Among all drugs, antibiotic is the main risk factor in CDI. Others like proton pump inhibitors, immunosuppressive agents and cancer therapy are also significant risk factors.

(i) Antibiotic

Clindamycin was the first antibiotic to be associated with CDI but now all classes of antibiotic apart from aminoglycoside have been implicated as a risk factor for CDI with the greatest risk being attributed to second and third generation cephalosporin (Owen *et al*, 2008). The most common antibiotics that predispose to CDI include clindamycin, penicillin group or cephalosporin and recently fluoroquinolones either used alone or combination has been associated with CDI. A case-control study by Loo *et al* (2005) in Quebec found an odd ratio of 3.9 for receipt of fluoroquinolones in the development of CDI and an odd ratio of 3.8 for cephalosporins.

The disruption of the microflora in the intestine by anitibiotics may increase the risk of CDI during therapy and for the days to weeks required for the intestinal flora to return to normal level (Owens *et al,* 2008).

There are three broad categories by which antibiotics affect CDI which are :

1. Certain agents like ceftriaxone which disrupt the intestinal flora and lack of significant activity against *C. difficile* promoted CDI during treatment.
2. Antibody with inhibitory activity against *C. difficile* like oral vancomycin may prevent colonization during therapy but such agents may facilitate colonization if exposure occurs during the period of microflora recovery.
3. Antibiotics which cause minimal disruption of the anaerobic microflora like aztreonam, did not promote CDI. Nevertheless antibiotics which cause relatively minor disruption of the anaerobic microflora like ciprofloxacin, have been associated with CDI (Owen *et al*, 2008)

Most patients with CDI were exposed to antibiotics several weeks to several months before diagnosis but it is important to be aware that disease onset can be shortly after antibiotic exposure. A report by Crabtree *et al*, 1999 showed that patients who received systemic antibiotics, the average time from completion of antibiotic to the development of CDI was 7 ± 2 days. Another study by Olson *et al*, 1994 reported that 96% of patients with symptomatic CDI had received antibiotics within 14 days before the onset of diarrhea and that all had received antibiotics within the previous three months.

Use of multiple antibiotics has been associated with an increased risk of CDI and in a case control study by Gerding *et al*, 1986, they found that patients who develop CDI

patients (Anand A *et al*, 1993). Chemotherapeutic agents such as adriamycin, cyclophosphamide, methotrexate and 5-fluorouracil have the capacity to precipitate this disease (Cudmore *et al*, 1982). Fulminant form of CDI has also been described in lung transplant recipients exposed to high dose immunosuppression and repeated courses of antimicrobials because of frequent pulmonary infections (Dallal RM *et al*, 2002). Several reports pointed to the risk of CDAD associated with the use of tacrolimus (Sharma A K *et al*, 1998). The powerful immunosuppressive action of tacrolimus presumably is the reason for this association. Immunocompromised patients are more susceptible to develop infections with *C. difficile*, and in those patients CDI tends to have also a poorer outcome. It is due to the faulty immune response to the toxin and unable to mount effective IgG antibody response against *C. difficile* that predispose to the development of symptomatic *C. difficile* disease in patients receiving immunosuppressive therapy. Kumar *et al*, 2004 reported that 32.7% of patients treated with methotrexate or mesalamine for psoarisis were tested positive for *C. difficile* toxin.

Administration of cancer therapy possessing antibacterial properties may also result in sufficient disturbance of intestinal microflora to allow colonization with *C. difficile*. A study by Emoto *et al*, 1996 reported that 6.1% of patients receiving cisplatin based combination chemotherapy for ovarian malignancy developed severe *C. difficile* – associated diarrhea.

(b)Age

CDI commonly occurs in the elderly group. During an outbreak in 2002 in Quebec, the frequency of *C. difficile*-associated diarrhea among persons more than 65 years old was ten-fold higher than observed in younger patients. In a study by Henrich *et al*, 2009 they reported that age more than 70 years old is associated with severe *C. difficile*-associated diarrhea. The reason for this association is uncertain and it may be due to the host factor such as diminished immune response to infection and elderly also has other co-morbidities that increases their risk to infection.

(c) Underlying disease

The Agency for Healthcare Research and Quality (AHRQ) found that hospitalized patients with *C. difficile*-associated diarrhea had over 10 diagnoses versus only six diagnoses among patients without *C. difficile*-associated diarrhea (Perry H *et al*, 2009). According to AHRQ data, four out of 20 most common diagnoses observed with CDI are sepsis, pneumonia, urinary tract infection and skin infection where antibiotic use would be difficult to avoid (Perry H *et al*, 2009).

Patients with inflammatory bowel disease (IBD) are also at greater risk in developing CDI than the general population. In a retrospective study done by Issa *et al*, 2007 they found that the rate of CDI had increased from 1.8% of IBD patients in 2004 to 4.6% in 2005 ($p< 0.01$). The association of IBD and CDI may be due to a variety of factors including antibiotics, hospitalization and many of the patients are using

immunosuppressive agent. Other medical conditions that has been associated with CDI include renal failure and chronic obstructive airways disease.

Severity of underlying disease has been shown to be a risk factor for *C. difficile* diarrhea (Mc Farland LV *et al*, 1990). This is probably because those patients with severe underlying disease will have prolonged hospital stay and they are more likely to be exposed to *C. difficile* and receive antibiotic therapy (Lorraine K *et al*, 2002).

(d) Hospitalization

Hospitalization and an increased length of stay in hospital have been identified as risk factors for CDI (Barbut *et al*, 2001). For patients who were hospitalized for two weeks or less, the rate of acquisition of *C. difficile* is approximately 13% and for patients with hospital stay of longer than four weeks the acquisition rate is 50% (Clabots *et al*, 1992).

In a study by Mc Farland *et al* 1989, specimens of surfaces from hospital rooms and hands of health care workers were obtained to detect evidence of contamination and almost half of the rooms with patients having symptoms of CDI showed an increased rate on environmental contamination. In patients with asymptomatic infection, the rate of contamination with *C. difficile* organisms in the room was 29% and for patients with no infection, the rate was 8%. From this study also *C. difficile* was detected on the hands of 59% of health care personnels caring for patients with infections. This result is

alarming as asymptomatic patients will be more likely to be in the group with uninfected patients and such arrangement increases the likelihood of horizontal transmission. Other risk factors for CDI include previous gastrointestinal surgery and Ryle's tube feeding.

1.3.4 CLINICAL FEATURES

The clinical manifestations of infection with toxin-producing strains of *C. difficile* can range from asymptomatic carriage to mild or moderate diarrhea to fulminant and sometimes fatal pseudomembranous colitis (Stuart *et al*, 2010).

(a) The carrier state

Asymptomatic carriage of *C. difficile* is quite common in hospitalized patients with about 20% of hospitalized adults being *C. difficile* carriers and in long term care facilities the carriage rate may approach 50% (Lawrence 2007). This occurs when patients are colonized with *C. difficile* organism but with no clinical symptoms even if there were colonized with toxin producing *C. difficile*. Asymptomatic carriage can be predicted by taking into account certain clinical factors such as recent antibiotic exposure or previous occurrence of CDI and these patients usually have good serum IgG response to *C. difficile* toxin. (Vaishnavi, 2010). They will shed the pathogen organism and have the potential to contribute significantly to disease transmission in long term care facilities.

(b) Symptomatic

It is usually mild to moderate diarrhea and sometimes accompanied by lower abdominal cramp. Symptoms usually occur during or shortly after antibiotic therapy but it can be delayed for several months (Owens *et al*, 2008). *C. difficile* toxin can be

detected from fecal specimens and the endoscopic findings and histologic features may be normal.

C. difficile colitis is the most common clinical manifestation of CDI without pseudomembranous formation. This is more serious than simple diarrhea and patients present with anorexia, malaise, nausea, abdominal pain and watery diarrhea. Dehydration and peripheral leukocytosis may be present. A study by Bulusu *et al*, 2000 showed that 60% of patients with unexplained leukocytosis had a positive stool test for *C. difficile* toxin compared to controls. The findings under sigmoidoscopy will be nonspecific with diffuse or patchy erythematosus colitis without pseudomembranous.

Pseudomembranous colitis (PMC) is the full blown of *C. difficile* colitis and it presents with more severe symptoms. The classic features of pseudomembranous can be observed under direct sigmoidoscopy examination which will reveal raised yellow plagues ranging from 2-10 mm in diameter scattered over the colorectal mucosa (figure 1). Higher total white blood count and hypoalbuminaemia (< 30mol/dl) may be observed in severely ill patient. Patients with hypoalbuminaemia may have ascites and it can be the only presenting manifestation of PMC (Vaishnavi, 2010).

Patient with fulminant colitis will present with severe lower quadrant or diffuse abdominal pain, diarrhea or abdominal distension. Diarrhea can be minimal in patients with ileus and severe protein losing enteropathy will result in hypoalbuminaemia. Toxic megacolon can occur and patients will have symptoms of severe toxicity which

include high grade fever, dehydration and markedly high total white count. Plain abdominal radiograph may show dilated small bowel with air-fluid level and this can lead to bowel perforation (figure 2).

Recurrent CDI is a difficult clinical problem and treatment may not be able to suppress *C. difficile* overgrowth. Two likely factors that increases the risk of recurrent CDI are an inadequate immune response to *C. difficile* toxins and persistent disruption of the normal colonic flora (Stuart J, 2009). It has been estimated that about 15-20 % of patients treated for CDI, relapse after successful treatment (Kelly CP, 1994). It occurs with the re-appearance of symptoms within one week of stopping treatment with either vancomycin or metronidazole. Patients can be re-infected with the same or different strains.

(c) Extracolonic manifestation

Besides large bowel, infection in the small bowel has also occurred with formation of pseudomembrane. It occurs more frequently in IBD patients as reported by Navaneetham *et al*, 2009 that small bowel involvement is more frequently in IBD patients who had undergone total colectomy or in patients with ileal-anal anastomosis.

CDI is also known to cause bacteremia with about 20 % mortality (Daruwala C, 2009),

and there was also case report by Libby *et al*, 2009, where monomicrobial *C. difficile* bacteremia in a young woman with underlying haematological malignancy but without any gastrointestinal symptoms.

Reactive arthritis which related to *C. difficile* involving joints of knee and wrist occurs in about 50 % of cases (Birnbaum *et al,* 2008). The symptoms begin at an average of 11.3 days after the onset of diarrhea and takes about an average of 68 days to resolve (Jacobs *et al*, 2001). CDI can also manifest in soft tissue infections like cellulitis, nectotizing fasciitis, skin infections and it can also occur in prosthetic device, intra-abdominal abscess, empyema and osteomyelitis (Birnbaum *et al*, 2008).

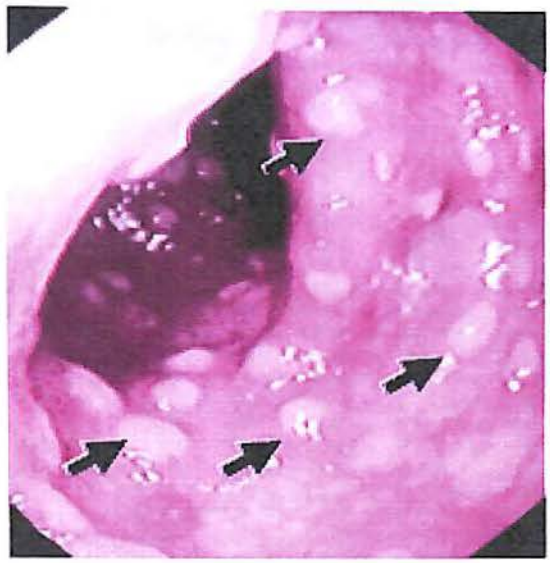

Figure 2. Pseudomembranous colitis. Endoscopic en face view of colon wall demonstrating several pseudomembranes (arrows). (From Brian W *et al,* 2002, Courtesy of Jonathan Leighton, MD, Division of Gastroenterology, Mayo Clinic Scottsdale, Scottsdale, Ariz.)

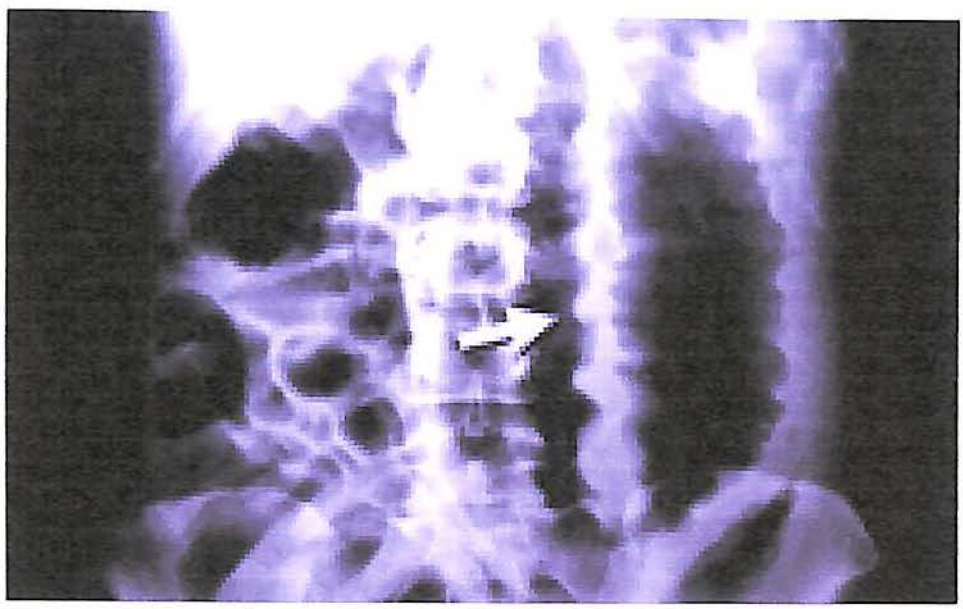

Figure 3. Acute toxic megacolon in a patient with fulminant pseudomembranous colitis. Note the thickened and edematous bowel wall (arrow). (From Brian W *et al*, 2002)

1.3.5 DIAGNOSIS (Table 1)

Accurate diagnosis is crucial in the management of CDI. Delaying in diagnosis may be due to the fact that CDI can mimic the more common benign antibiotic- associated diarrhea that is not caused by *C. difficile*. From original observations, *C. difficile* toxins are responsible for most of antibiotic-associated diarrhea, therefore most diagnostic tests that have been developed were to detect toxin A and or toxin B produced by *C. difficile* in the stool. There are various laboratory investigations that can be used to detect toxins and each test has their own advantages and disadvantages.

(i) Enzyme immunoassay

Enzyme immunoassays (EIAs) for *C. difficile* toxins in the stool have been the most frequently used because they are easy to use and provide quick result. It can detect both toxin A and B but it is less sensitive.

(ii) Glutamate dehydrogenase test

Glutamate dehydrogenase (GDH) test only detect a common antigen and not a toxin and provide no information regarding toxigenicity of the isolate. This test can be used as a screening test where a positive test will require further testing to determine whether *C. difficile* strain is toxigenic or not. A negative test will be considered negative for the pathogen.