PROSPECTIVE OBSERVATIONAL STUDY TO ASSESS PATIENT PROFILE WITH CLOSTRIDIUM DIFFICILE COLITIS

Thesis submitted in partial fulfillment of the requirements for the Degree of Master of Clinical Pharmacy And Pharmacy Practice

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CERTIFICATION OF APPROVAL

This is to certify that MR. SALMAN MONDAL bearing class Roll No- 001711403006 , Examination Roll No-**M4PHC19006**, Registration No- **140860 of 2017-2018**, a candidate of Master of Pharmacy in Clinical Pharmacy and Pharmacy Practice, has submitted the project thesis entitled "PROSPECTIVE OBSERVATIONAL STUDY TO ASSESS PATIENT WITH CLOSTRIDIUM DIFFICILE COLITIS IN ICU " under our supervision at AMRI Hospitals, Dhakuria , Kolkata for partial fulfillment of requirement for the completion of M.Pharm.in Clinical Pharmacy and Pharmacy Practice".

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AMRI HOSPITALS DHAKURIA

Ref: AMRI -EC/AP-03/2017-18 **Date:04.10.18**
Dr.Mahuya Bhattacharya
Subject: Ethics review of clinical research proposal

Protocol title: "A prospective observational study to assess patient profile with clostridium difficile colitis"
Study Site: AMRI Hospitals, Dhakuria.

Name of Student: Salman Mondal
Dear Dr. Bhattacharya,
In the meeting held on **04.10.18**, the members of the ethics committee reviewed and discussed the protocol and other related documents of the above mentioned study:

The following members of the committee were present:
Prof. Santanu Kumar Tripathi (Chairman)
Dr. Arghya Majumdar (Member Secretary)
Mr. Atanu Ray Chaudhuri (Legal Advisor)
Dr. Avijit Bhattacharya (Clinician)
Dr. Subhra Dhar (Basic Medical scientist)
Dr.Ashok Kr Mondal (Clinician)
Ms.Anjali Ghosh (Social Scientist)
Ms. Sunita Sarkar (Community Representative)
Ms. Atashee Chatterjee Sinha (Philosopher)

The Thesis protocol, Data Collection Proforma were submitted and duly reviewed by Members of IEC present in the meeting:

(1) **Approval is hereby granted to the above mentioned study protocol and related documents in order to conduct the study in AMRI Dhakuria.**

(2) **The Committee should be informed**:
(a) about the progress of the study every six months
(b) about any Serious Adverse Events occurring in the course of the study within 24 hours of their occurrence, including their management and compensation etc
(c) about any changes in the protocol and patient information/informed consent documents.

(3) **The final report of the study shall have to be submitted to the IEC in all cases, even when the study is abandoned for any reason.**

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Contents

INTRODUCTION

Clostridium Difficile - Bacteriology

Clostridium difficile(CD) was first detected by Hall and O'Toole in 1935 as a component of the normal stool flora of new-born infant[1]. For the next 40 years, there were infrequent reports of *C. difficile* isolation, with few findings implying that this organism could cause disease. However, in 1978, *C. difficile* was identified as the primary cause of pseudomembranous colitis[2,3]. It was difficult to culture this organism in the laboratory during that time; hence, it was named B. difficile[1,2,3] . CD is an established human and animal pathogen that primarily causes gastroenteritis. CD is a gram positive, anaerobic, spore-bearing bacillus present as a common inhabitant in contaminated environments.[3,4]. Figure 1 shows the structure of a CD bacteria.

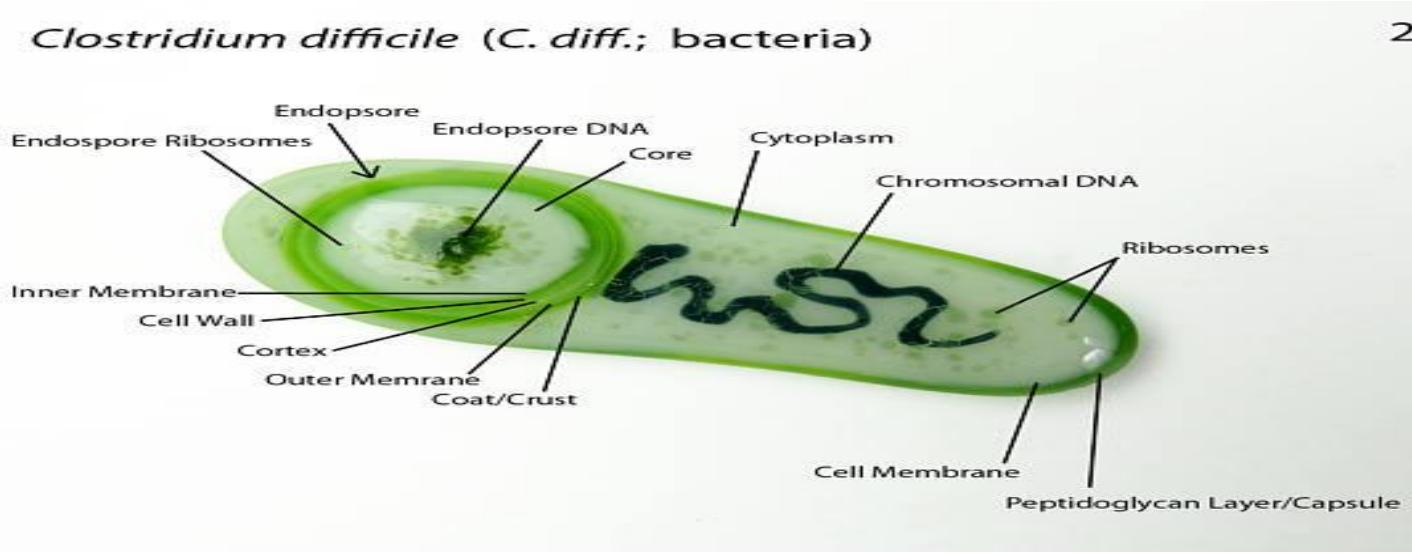


Figure 1 showing the structure of a CD bacteria.

The C.D bacterium has two forms, an active, infectious form that cannot survive in the environment for prolonged periods, and an inactive, "noninfectious" form, called a spore (1) which can survive in the environment for prolonged periods. Although spores cannot cause infection directly, when they are ingested they transform into the active, infectious form (5). *C. D* spores are found frequently in hospitals, nursing homes , extended care

facilities, and nurseries for newborn infants(3,5).C.D can be found on -bedpans, furniture, toilet seats, linens, telephones, stethoscopes, fingernails, rings(jewelry), floors, infants room(1).

Clostridium difficile associated colitis (CDAC) is an infection of the colon by the bacterium, *C.D* (C.difficile) (5). *C. D* causes colitis by producing toxins that damage the lining of the colon. Serious complications of *C. D* colitis include dehydration, rupture of the colon, and spread of infection to the abdominal cavity or body [1,5]. Severe infection is life-threatening. Patients with mild *C.D* colitis may have a low-grade fever, mild diarrhea (5-10 watery stools a day),mild abdominal cramps and tenderness. Patients with severe *C.D* colitis may have a high fever of 102 F to 104 F (39 C to 40 C), severe diarrhea (more than 10 watery stools a day) with blood, and severe abdominalpain and tenderness[5].

CDtoxin **A and B** are toxins generated by *Clostridium difficile*[6]. The toxins are the main virulence factors produced by the gram positive, anaerobic, *Clostridium difficile* bacteria. The toxins function by damaging the intestinal mucosa and cause the symptoms of *C.D* infection, including pseudomembranous colitis[5,6].

TcdA is one of the largest bacterial toxins known. With a molecular mass of 308 kDa, it is usually described as a potent enterotoxin [7], but it also has some activity as a cytotoxin[8]. The toxin acts by modifying host cell GTPase proteins by glucosylation, leading to changes in cellular activities[9]. CD Toxin B is however similar to CD Toxin A.

Toxins delivery into the host cell cytosol can be divided into seven main steps: [11] toxin binding to the host cell surface receptor; [13] toxins internalization through a receptor-mediated endocytosis; [16] endosome acidification; [13] pore formation; (10) GTD release from the endosome to the host cell cytoplasm; [12] Rho GTPases inactivation by glucosylation; and downstream effects within the host cell, *i.e.*, toxins-induced cytopathic and cytotoxic effects[14] .Figure 2 shows how CD toxins induced cytopathic and cytotoxic effect[14].

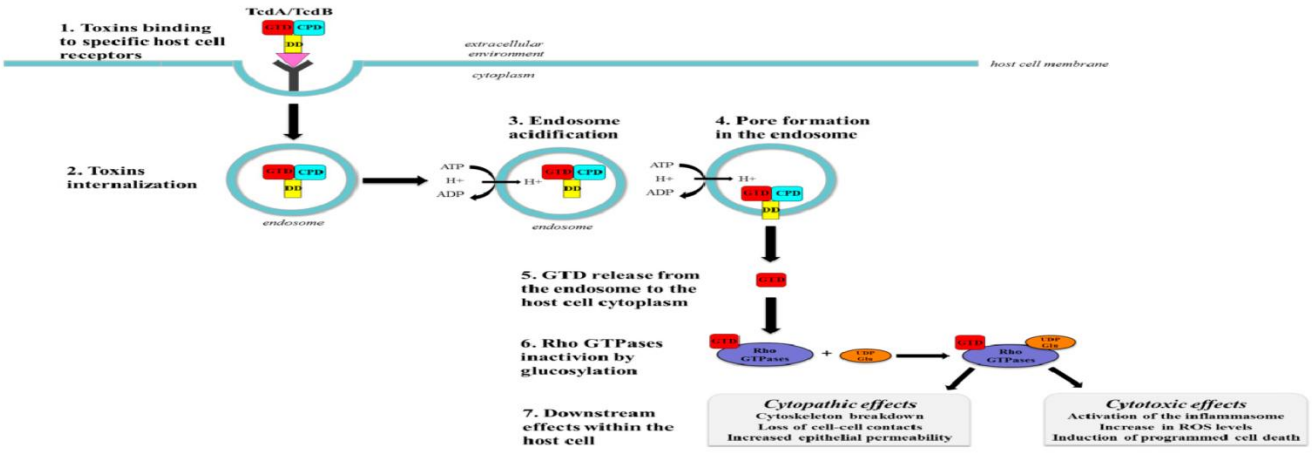


Figure 2 shows how CD toxins are delivered to the host cell. (Color code: GTD: N-terminal glucosyltransferase domain (red) ; CPD: cysteine protease domain (cyan); DD: delivery domain (yellow) [10,11].

Clostridium difficile associated colitis

Clostridium difficile is the most important nosocomial pathogen recognized globally as an enteric pathogen responsible for antibiotic-associated diarrhea (AAD) and colitis[17]. It is a significant cause of morbidity and mortality among hospitalized patients, and the incidence of C.D infection (CDI) has dramatically increased due to frequent

usage of broad-spectrum antibiotics in these patients[18]. Recent reports estimate the prevalence of CDIs as 20%–30% and in India as 15%–20% in patients taking antibiotics[17,18]. CDI is associated with a mortality rate of 25% in the elderly people[18]and at present, the all-cause mortality at 30 days is found to be 15% or greater[19]. Among all the risk factors involved, antibiotics are the most important risk factor. Other drugs such as immunosuppressive agents, proton pump inhibitors and cancer therapeutics are also significant risk factors for CDAC precipitation[19].

Antibiotic-associated CD colitis - is an infection of the colon caused by *CD* commonly acquired during hospital stays, infecting approximately 1% of patients admitted to hospitals in the United States [5]. More than half a million *C. difficile* infections occur in hospitals in the US each year, with about 300,000 occurring while in the hospital or shortly after hospitalization [5]. Studies report that even after a stay of only two days in a hospital, 10% of patients will develop infection with C.D [16]. *C.D* also may be acquired outside of hospitals in the community. It is estimated that about 200,000 infections with *C. difficile* occur in the community unrelated to hospitalization each year in the U.S [5,18].

C. difficile spores lie dormant inside the colon until a person takes an antibiotic[5]. The antibiotic disrupts the other bacteria that normally are living in the colon and preventing *C.D* from transforming into its active, disease-causing bacterial form. As a result, *C.D* transforms into its infectious form and then produces toxins (chemicals) that inflame and damage the colon[5,6]. The inflammation results in an influx of white blood

cells to the colon[5]. The severity of the colitis can vary. In the more severe cases, the toxins kill the tissue of the inner lining of the colon, and the tissue falls off[16]. The tissue that falls off is mixed with white blood cells (pus) and gives the appearance of a white, membranous patch covering the inner lining of the colon. This severe form of *C.D* colitis is called pseudomembranous colitis because the patches appear like membranes, but they are not true membranes[19]. Not everybody infected with *C.D* develops colitis [5].Many infants and young children, and even some adults, are carriers (they are infected but have no symptoms) of C. difficile. *C.D* does not cause colitis in these people probably because (a) the bacteria stay in the colon as non-active spores, and (b) the individuals have developed antibodies that protect them against the *C.D* toxins[5].

Drugs causing CD Colitis

(1) Antibiotics - Although the antibiotic clindamycin has been widely recognized as causing C.D colitis, many commonly prescribed antibiotics also cause colitis[19]. Examples of antibiotics that frequently cause *C.D* colitis include: ampicillin, amoxicillin, and cephalosporin[5]. Antibiotics that occasionally cause *C.D* colitis include: penicillin, erythromycin, trimethoprim and quinolones such as ciprofloxacin. Rarer antibiotics that cause *C.D* colitis include: tetracycline, and amino glycosides[20]. While most *C. difficile* colitis in the US is caused by antibiotics, *C.D* colitis also can occur in patients without exposure to antibiotics[5]. For example, patients with ulcerative colitis have been known to C.D colitis without exposure to antibiotics [6].

(2) Acid suppressive agents: Gastric acidity constitutes a major defense mechanism against ingested pathogens, and loss of the normal stomach acidity has been associated with colonization of the normally sterile upper gastrointestinal tract[21]. Acid suppressive agents such as proton pump inhibitors and H2-receptor antagonists (H2RAs) increase gastric pH and proton pump inhibitors have also been shown to affect leukocyte function,[21,22] which may contribute to the reported associations with an increased risk of respiratory tract infections[23]and enteric infections[24] including hospital and nursing home acquired CDAC[25,26].

Proton Pump Inhibitors: Colonization of normally sterile upper gastrointestinal tract can be a consequence of gastric acid suppressive use due to raised pH of stomach resulting in increased risk of enteric infections including CDAC[27]. Gastric acid secretion acts as a barrier for enteric pathogens[28]. Proton pump inhibitors (PPI) inhibit the gastric acid secretion by interfering with the activity of H+ /K+ -ATPase of the parietal cells and may thus contribute to the pathogenesis of CDAC by altering the intestinal flora[5]. The total volume of prescribing for PPI increased 10-fold in the United Kingdom between 1992 and 1995[28] . PPI use was a significant risk factor for CDAC in a retrospective case control study[28]. In a similar study, Cadle et al.[29] found that PPI therapy was associated with an increased risk of recurrent colitis due to C. difficile, Jayatilaka et al,[30] in a five year study period found that PPI usage correlated exactly with the overall annual increased CDAC incidence and believed that the widespread prescription of PPI could be responsible . Nachnani et al., [31], reported that PPI therapy was independent and the only risk factor associated with an increased length of hospital stay in CDAC patients. Lowe et al.,[27] , also notified an association between PPI therapy and

hospitalization for community-acquired CDAC among elderly patients treated with broad-spectrum antibiotics[28]. Proton pump inhibitor may therefore be considered a risk factor for CDAC because of (i) the survival of spores facilitated by elevated gastric pH levels and (ii) due to the effect of PPI on immune function or on the toxin production of the organism[27,28]. Inhibition of gastric acid removes a defence against ingested bacteria and spores, increasing the risk of some forms of gastroenteritis[28]. The risk of CDAC in hospitalized patients receiving antibiotics may thus be compounded by exposure to PPI therapy. Curtailing the inappropriate use of PPI therapy may help prevent the increased hospital stay by CDAC patients and reduce overall costs of management and therapy[28].

(3) **Immunosuppressive Agents**: Infection is the leading cause of morbidity and mortality in the early post-transplant period in patients taking immunosuppressive agents [29]. Immunosuppressive drugs have been reported to be associated with the development of CDAC[30,31]. Faulty immune response to C.D toxins has been noted as one of the major host factors predisposing patients to the development of symptomatic CDAC[33,34]. Patients at highest risk for fulminant disease include those who have recently received immunosuppressive therapy[35,36].The ability of the immune system of the host to produce protective antibodies against C.D toxins plays an important role in reducing the severity of disease and preventing further recurrences[36]. The host antibody response play a major role in disease outcome. Serum levels of IgG antibody against toxin A are found to be higher in patients with a mild CDAC than in those with prolonged or severe diarrhea[38]. Patients with C.D colonisation and a serum IgG response C.D enterotoxin usually become asymptomatic carriers while patients lacking protective immunity develop diarrhoea and colitis[37].Even if the immune

response to C.D toxins is inadequate, it will predispose patients to severe, prolonged and recurrent C.D diarrhoea[37]. Patients receiving immunosuppressive drugs are debilitated and therefore are unable to mount an effective IgG antibody response against C.D toxin A thereby increasing the risk for CDAC[37].Though the ability to mount an immune response is not protective against C.D colonisation, it is associated with decreased morbidity, mortality, and recurrence of C.D -associated diarrhoea[37].

(4) Corticosteroids: C.D colitis may occur without prior use of antibiotics in immunosuppressed patients[39]. Exposure to corticosteroids is significantly associated with an increased risk of CDAC relapse warranting a longer treatment course. C.D colonization is more frequent in intensive care and oncology units where broad spectrum antibiotics and immunosuppression are wide spread. Keven et al,[35], reported that 5.5% of patients after solid organ transplantation developed C.D colitis at a median of 30 days after transplantation. Dallal et al.,[36], reported 31% incidence of CDAC in lung transplant patients compared to 1.6% overall. Ulcerative colitis patients unresponsive to corticosteroids may require long time immunosuppressive treatment which may result in multiple infections, inclusive of C.D[38]. Diarrhoea is a common manifestation after liver transplantation and a side effect of immunosuppressive medication with C.D as one of the aetiologic agents[38].

(5) Cancer Therapeutics: Administration of cancer chemotherapeutic agents possessing antibacterial properties may also result in sufficient disturbance of the intestinal micro flora to allow colonisation with C.D[38,39]. This can occur without the associated use of antibiotics. Emoto et al,[39] reported severe CDAC in 6.1% of patients receiving cisplatin based combination

chemotherapy for ovarian malignancies. Resnik and Lefevre[40]described development of fulminant C.D colitis in a 66 year old patient with ovarian cancer who received paclitaxel and carboplatin chemotherapy. Kumar et al,[41] reported that 19 out of 58 patients treated with methotrexate or mesalamine for psoriasis were positive for C. difficile toxins.

Thus the combination of the environmental presence of C.D in health care settings and the number of people receiving antibiotics, immunosuppressives, proton pump inhibitor or cancer therapeutics in these settings can result in frequent outbreaks[40].

Other risk factors associated with CD:

(1) **Gastrointestinal diseases:** Identifiable risk factors involving gastrointestinal diseases are inflammatory bowel disease, bowel ischaemia, mechanical bowel cleansing, enteric infections that change colonic micro flora, prolonged presence of a nasogastric tube for enteral feeding, use of electronic rectal thermometers, use of enemas, gastrointestinal stimulants and stool softeners [39,41].

(2) **Renal impairment and uraemia:** Leading to renal failure is another established risk factor. Admission to dialysis ward in three months before index admission was found to be associated with CDAC[42].

(3) **Impaired Immunity (other than drugs):** Severely ill patients with compromised immune function are particularly susceptible to CDAC. Conditions that impair host-immune defenses

inclusive of irradiation, malnutrition, shock and use of chemotherapeutic agents are also potential risk factors[42].

(4) Prolonged Hospital Stay: Specific population appears to be at greater risk for CDAC than general population and this includes patients in nursing homes and those in hospital settings. The reason is that compared to the general population, these patients are older and receive more antimicrobials and gastric acid suppressive[41]. Thus most cases and outbreaks occur in health care settings and medical patients are at significantly increased risk than are surgical patients[42]. Table 1 demonstrates risk factors associated with CDAC.

Table 1 —*Risk Factors Associated With CDI(42,43).*

Variable	RISK Factors
Perturbation of the intestinal flora/mucosa or immune	Antibiotic treatment Fluoroquinolone-resistant BI/NAP1/027 Proton pump inhibitors and H 2 -receptor antagonists Chemotherapy Glucocorticoids Radiation treatment Intestinal stasis (medications) Abdominal surgery Nasogastric tubes and enemas system
Environmental contamination	Length of stay in hospital or long-term care facility Possible: food contamination, pets, and farm animals
Host factors	Age > 65 y Multiple comorbidities Peripartum women and children Inflammatory bowel disease HIV Chronic kidney disease requiring Hemodialysis

Diagnostic Modalities

Clostridium difficile associated diseases can be suspected and/or diagnosed clinically, endoscopically, radiologically as well as by identification of aetiological agent and by toxin assays[43]. A history of antibiotic use is important in the diagnosis of *C. difficile* colitis. Patients taking antibiotics (or recently having taken antibiotics) who develop abdominal pain, cramps and diarrhea are usually tested for *C.D* infection[43]. However, doctors do not always wait for the appearance of diarrhea to start testing for *C.D* since in rare instances *C.D*can cause abdominal pain and tenderness without diarrhea[5].

Patients with *C.D* colitis often have elevated white blood cell counts in the blood, and, in severe colitis, the white blood cell counts can be very high (20,000 to 40,000) [5]. Patients with *C. difficile* colitis also often have white blood cells in their stool when a sample of stool is examined under a microscope[5]. Elevated white blood cell counts and white blood cells in the stool, only demonstrate that there is colitis and not that the cause of the colitis is *C. difficile.* The most widely used test for diagnosing *C.D* colitis is a test that detects toxins produced by *C.D* in a sample of stool. There are two different toxins, toxin A and toxin B, both capable of causing colitis [5]. Accurate tests for both toxins are available commercially for use in all laboratories. Other tests such as flexible sigmoidoscopy and colonoscopy often are also necessary to look for pseudomembranes that are characteristic of *C.D* colitis[5].

Flexible sigmoidoscopy and colonoscopy

Flexible sigmoidoscopy is an examination in which a doctor inserts a flexible fiberoptic tube with a light and a camera on its end into the rectum and sigmoid colon[43]. (The sigmoid colon is the segment of the colon that is closest to the rectum.) Most patients with *C.D* colitis, will develop pseudomembranes in the rectum and the sigmoid colon[42]. Some patients with *C.D* colitis may have pseudomembranes only in the right colon (the segment of the colon farthest from the rectum). Patients with pseudomembranes confined to the right colon require colonoscopy in order to see the pseudomembranes[43]. Figure 3 shows endoscopic view of pseudomembranous colitis.

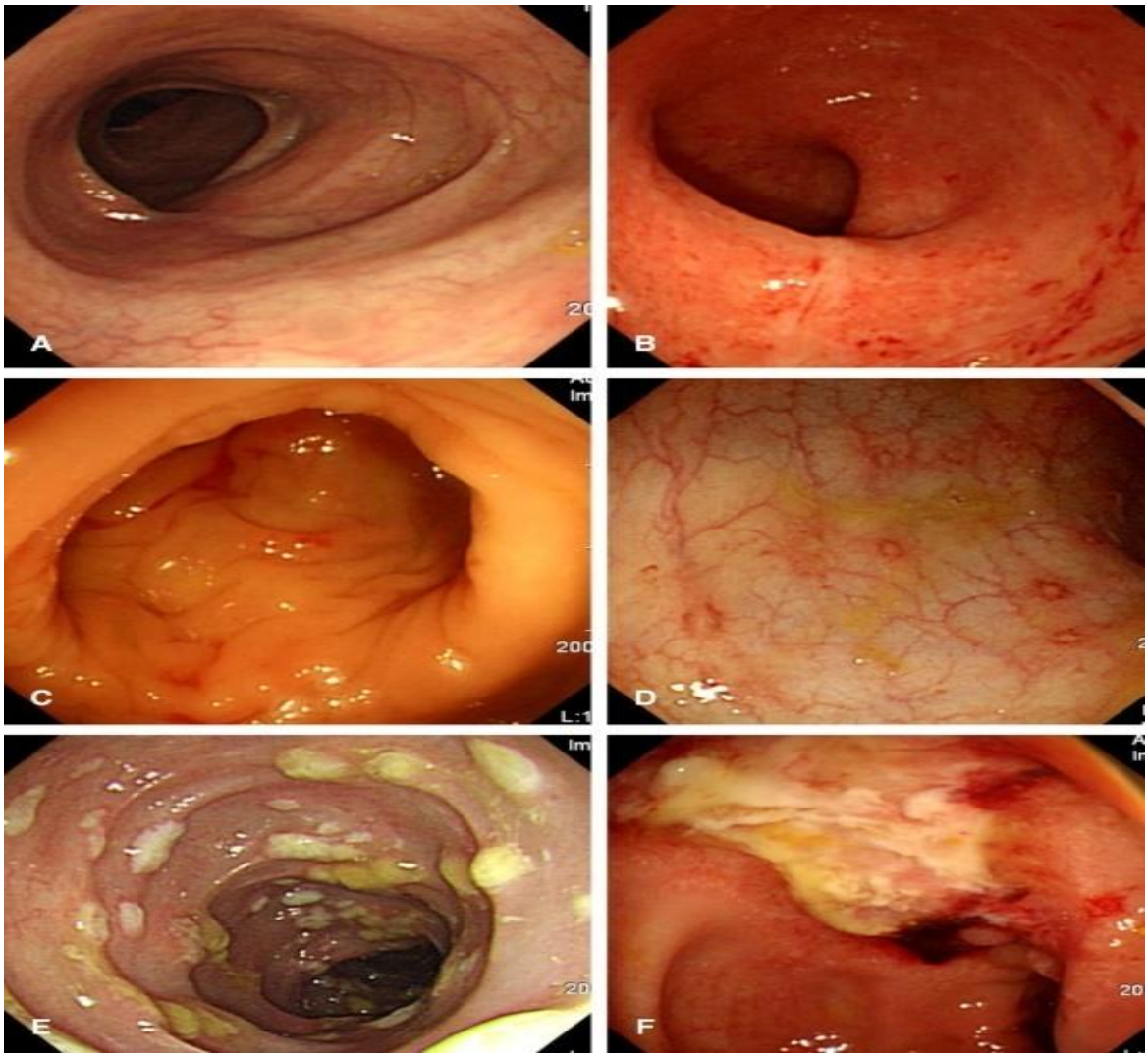


Figure 3:Endoscopic view of pseudomembranous colitis

X-rays

X-ray examinations and computerized tomography (CT) examinations of the abdomen will occasionally demonstrate thickening of the wall of the colon due to inflammation, but these X-ray findings also are non-specific[5].

I**mmunochromatography assay**

The immunochromatography technique is a single test enzyme immunoassay for detection of toxins A and B in faecal samples[5].

Enzyme immunoassays

Detection of either toxin A alone or both toxins A and B in stool specimens are being done with commercially available kits . Figures 4 demonstrate the ELISA pictures of CDAC diagnosis[43].

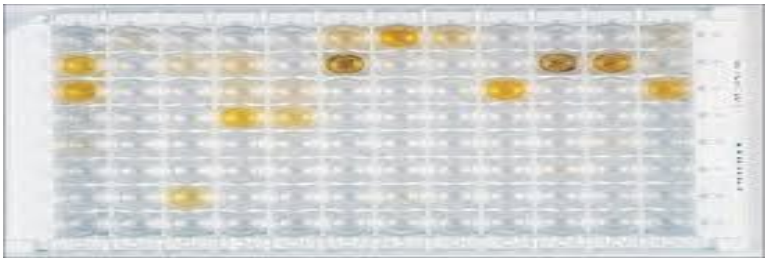


Figure 4 : Elisa test for C.difficile toxin

Treatment

Metronidazole and vancomycin are the commonest pharmacological treatment options for CDI. Metronidazole has high oral bioavailability, associated with reliably therapeutic colonic luminal exposure[46].These two antibiotics usually are taken orally for 10 days. Both antibiotics are equally effective. With either antibiotic, fever usually resolves in one or two days, and diarrhea in three or four days. Other antibiotics, that have been used effectively against *C.D* recently is Fidaxomicin[5].

The choice of which antibiotic to use depends on the individual patient's situation and the preferences of the treating doctor.[48] . Vancomycin is usually reserved for patients who do not respond to metronidazole, are allergic to metronidazole, or develop side effects from metronidazole. Vancomycin can also achieve much higher antibiotic levels in the colon than metronidazole [46]. However, Metronidazole still remains first-line treatment for non-severe CDI.[49]. Recent data suggest combination therapy (IV metronidazole plus oral vancomycin) may confer an additional survival benefit in the region of 20% for critically unwell patients over vancomycin monotherapy.[50]. The novel macrocyclic antibiotic Fidaxomicin has a narrow spectrum of activity. Fidaxomicin is around seven times the cost of oral vancomycin or 600 times the cost of metronidazole for a standard 10-day course[51]. However, detailed cost-effectiveness analyses, modelling various healthcare settings, suggest that fidaxomicin is cost-effective in severe disease and, particularly, for patients with high risk of recurrence[52]. This is attributed to reduced recurrence rates and decrease in spread of *C. difficile* spores. The recommended duration of therapy for CDI is 10–14 days and should be guided by clinical response. The licensed duration of fidaxomicin is 10 days. Intracolonic vancomycin may be considered in severe disease[50].

Intravenous immunoglobulin (IVIG) at a dose of 400mg/kg stat may be considered as salvage therapy in severe CDI.[50].The rationale for use is that IVIG is thought to bind to and neutralise toxin A[53].

Fecal microbiota (bacterial population) transplants are also becoming common for relapsing patients because of its success rates. Feces from non-infected donors are made into a suspension[50]. The source of the transplanted fecal microbiota can be healthy family members, acquaintances or from stool banks[53]. The fecal microbiota may be given by enema or by colonoscopy inserted into the rectum, by a feeding tube inserted through the mouth or nose into the upper small intestine, or by way of frozen capsules taken by mouth. The normal bacteria from the donor's stool displaces the *C. difficile* bacteria(53). FMT was first documented for use in pseudomembranous colitis in 1958,[54] but it was not widely adopted until recently. Figure 4 shows.C.D infection and role of faecal microbiota transplantation.

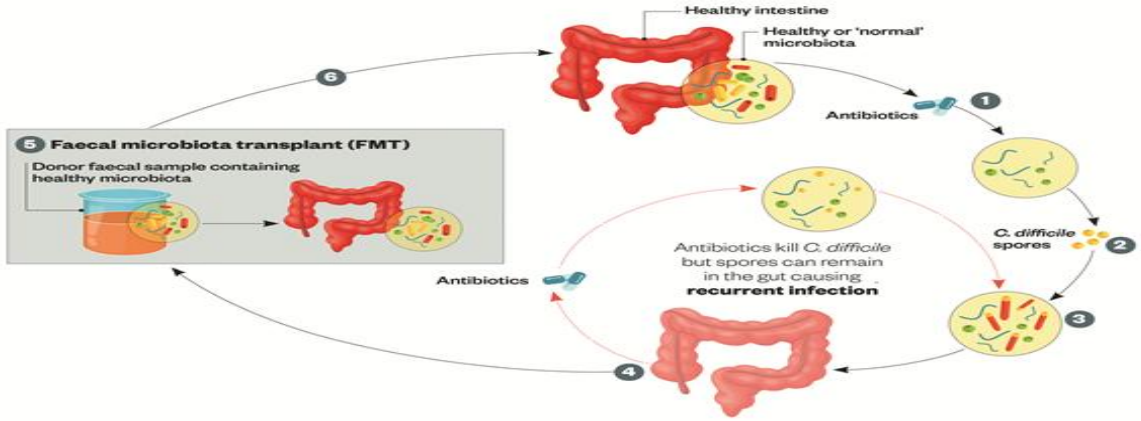


Figure 4 showing C.D infection and the role of faecal microbiota transplantation(54).

1) Ingesting antibiotics results in a reduction of microbial species and diversity.
2) Spore picked up from the environment are ingested.
3) The C.D spores germinate resulting in dysbiosis of the gut microbiota.
4) This can result in the development of CDI (characterized by severe diarrhea, abdominal pain, nausea and fever), Inflammation and cell death occurs due to presence of toxins. Severe CDI can cause pseudomembranous colitis.
5) Faecal microbiota transplant (FMT): The faecal samples is filtered and administered by enema, transcolonic infusion or nasoduodenal infusion.
6) The result is restoration of stable, healthy microbiota.(54,55)

Several measures significantly reduce the incidence of CDAC. The disease can be prevented and/ or controlled when following procedures are implemented:

1. Restriction on usage of antimicrobials, particularly for common ailments like cough, cold and sore throat, hand hygiene with soap and water or with alcohol based products,
2. Using gloves and gowns to avoid contact with patients, their body wastes and their environment, Avoiding the use of electronic thermometers whose handles can get contaminated,
3. Isolated patients should have patient care items and equipments dedicated to them,
4. Repeated testing of stool in CDAC patients should be discouraged unless and until symptoms resolve with treatment.
5. Healthcare workers should be educated about risk factors and infection control measures to prevent the spread of CDAC[56].

LITERATURE REVIEW

Clostridium difficile *associated* colitis (CDAC) is an infection of the colon by the bacterium, *C.D* (C.difficile). *C. D* causes colitis by producing toxins that damage the lining of the colon. Serious complications of *C. D* colitis include dehydration, rupture of the colon, and spread of infection to the abdominal cavity or body. Antibiotics are the most important risk factor. Other drugs such as immunosuppressive agents, proton pump inhibitors , cancer therapeutics and critically ill patients are also significant risk factors for CDAC precipitation. Related literature search has been done from Pubmed and Google scholar. Literature that has helped to design my study, and provided with background information were included in this chapter of the term paper.

Emoto et al., (1996), conducted a study on Clostridium difficile colitis associated with cisplatin-based chemotherapy in ovarian cancer patients. They studied that administration of cancer chemotherapeutic agents possessing antibacterial properties may also result in sufficient disturbance of the intestinal micro flora to allow colonisation with C. diffcile. This can occur without the associated use of antibiotics. They reported severe CDAD in 6.1% of patients receiving cisplatin based combination chemotherapy for ovarian malignancies[39].

Bliss et al.,(1998), a study conducted on Acquisition of Clostridium difficile and Clostridium difficile-associated diarrhea in hospitalised patients receiving tube feeding. They studied the incidence of C. difficile acquisition and CDAD in tube-fed and non tube-fed patients and reported that tube-fed patients, especially those receiving post pyloric tube feeding are at greater risk for development of CDAD than are hospitalised, non-tube-fed patients[72].

Vaishnavi et al.,(1999), Conducted a study on Detection of Clostridium difficile toxin by an indigenously developed latex agglutination assay. Like Wong et al.,(2002), they also reported that Clostridium difficile is the aetiological agent for almost all cases of pseudo membranous colitis and 15-25% of antibiotic associated diarrhoea. In recent years, C. difficile associated disease (CDAD) has been increasing in frequency and severity due to the emergence of virulent strains. They concluded that 30% positivity for C. difficile toxin in hospitalised patients of all age group receiving single to multiple antibiotics for various ailments, but only in seven per cent of samples from patients not receiving antibiotics. When only adult population were investigated, the positivity for C. difficile toxin was 19.4% in the antibiotic receiving hospitalised patients[17].

West et al.,(1999), conducted a study on Clostridium difficile colitis after kidney and kidney-pancreas transplantation. They stated that Leading to renal failure is another established risk factor. Admission to dialysis ward in three months before index admission was found to be associated with CDAD and investigated to find whether immunosuppressed transplant recipients were more prone to CDAD and its complications and observed an increased incidence of C. difficile colitis in paediatric kidney-pancreas recipients. They reported overall eight per cent incidence of CDAD with 16% in the paediatric kidney group and 15.5% in the kidney-pancreas group[33].

Kyne et al., (2001), attened a study on Association between antibody response to toxin A and protection against recurrent Clostridium diffcile diarrhoea. They studed that the Gram-positive anaerobic bacterium *Clostridium difficile* produces toxins A and B, which can cause a

spectrum of diseases from pseudomembranous colitis to *C. difficile*-associated diarrhea. A limited number of *C. difficile* strains also produce a binary toxin that exhibits ADP ribosyltransferase activity. They reported an association between defective humoural response to toxin A and a more severe form of C. difficile infection. Acute inflammatory infiltration occurs in the colonic mucosa due to the destruction of epithelial cells with increased permeability of the tight junctions. This leads to fluid secretion, inflammation and mucosal damage leading to diarrhea[37].

Katyal et al.,(2002), conducted a study on excretion of brush border membrane enzymes in patients with Clostridium difficile infection. They studied that Clostridium difficile is the aetiological agent for almost all cases of pseudo membranous colitis of antibiotic associated diarrhoea. In recent years, C. difficile associated disease (CDAD) has been increasing in frequency and severity due to the emergence of virulent strains . They reported a significant disturbance in the intestinal brush border enzymes in patients with C. difficile diarrhea[71].

Dallal et al.,(2002), conducted a study on Fulminant Clostridium difficile reported 31% incidence of CDAD in lung transplant patients compared to 1.6% overall[36].

In a study by Wong et al.(2002), on Colorectal disease in liver allograft recipients, Clostridium difficile was reported as the aetiological agent for almost all cases of pseudo membranous colitis and 15-25% of antibiotic associated diarrhoea. In recent years, C. difficile associated disease (CDAD) has been increasing in frequency and severity due to the emergence of virulent strains. They also reported that C. difficile and medication were the commonest

colorectal cause of morbidity after orthotopic liver transplantation in addition to ulcerative colitis and cytomegalovirus infection[57].

Kumar at al., (2004), conducted a study on Clostridium difficile toxin assay in psoriatic patients.Like Vaishnavi et al.,(1999), They also reported that the C. difficilepathogenicity locus (PaLoc) consists of TcdA and TcdB and three additional genes, negative (tcdC), positive (tcdD) regulators as well as a holin-like pore-forming protein (tcdE) .They concluded that 19 out of 58 patients treated with methotrexate or mesalamine for psoriasis were positive for C. difficile toxins[41].

Keven et al.,(2004), in their study looked at C.D colitis risk factor in patients after kidney and pancreas-kidney transplantation. They reported that 5.5% of patients after solid organ transplantation developed C. difficile colitis at a median of 30 days after transplantation[34].

McDonald et al.,(2005), conduct a study on An epidemic, toxin genevariant strain of Clostridium difficile. They stated that Clostridium difficileis the major etiological agent of nosocomial diarrhea primarily precipitated by antimicrobial therapy. They found strain in eight institutions, in six different states in the United States and it represented more than 50% of the isolates from five institutions. This strain has also been reported from Great Britain, The Netherlands and Belgium[70].

Al-Tureshi at al.,(2005), conducted a study on Albumin, length of stay and proton pump inhibitors.They studied that Colonization of normally sterile upper gastrointestinal tract can

be a consequence of gastric acid suppressive use due to raised pH of stomach resulting in increased risk of enteric infections including CDAD. They concluded that low albumin level, a recent admission to a nursing facility, and the use of PPI are the important risk factors for CDAD while assessing institutional patients with diarrhea[69].

Pepin et al.,(2005), Conducted a study on Emergence of fluoroquinolones as the predominant risk factor for Clostridium diffcile-associated diarrhoea.They studied that increased incidence of nosocomial CDAD with marked increase in severity of cases requiring colectomy or ending in death. This was attributed to increased use of fluoroquinolones particularly levofl oxacin, though clindamycin and ceftriazone were also identified as risk factors.They reported that elevated risk of CDAD with PPI occurred in univariate analysis but not after adjustment for co-morbidities on multivariate analysis[68].

Loo et al.,(2005), In their study on Clostridium difficile-associated diarrhea with high morbidity and mortality.They reported that CDAD patients showed a higher mortality, with cephalosporins and fluoroquinolones as risk factors[67].

Lowe et al.,(2006), conducted a study on Proton pump inhibitors and hospitalization for Clostridium difficile associated disease. Like that Al-Tureshi at al.,(2005), They studied that a higher number of cases involving toxic mega colon, colectomy or death. Among all the risk factors, inclusive of the host and the environmental factors, antibiotics are the most important ones. Surgical patients comprise 55-75% of all patients with CDAD due to the fact that perioperative prophylaxis requires the use of antibiotics. Thus CDAD is a growing nosocomial

and public health challenge. They found an association between PPI therapy and hospitalisation for community-acquired CDAD among elderly patients treated with broad-spectrum antibiotics[27].

Pudhota et al.(2006), conducted a study on detection of Clostridium difficile pseudomembranous colitis in the absence of diarrhoea with an early use of endoscopy in elderly patients. They studied that colonoscopy is an examination in which a doctor inserts a flexible fiberoptic tube with a light and a camera on its end into the rectum and sigmoid colon. (The sigmoid colon is the segment of the colon that is closest to the rectum). They reported that most patients with *C. Difficile* colitis, will develop pseudomembranes in the rectum and the sigmoid colon[45].

Si-Wook et al.,(2007), conducted a study on Clinical aspects of rifampicin-associated pseudomembranous colitis. They studied that the antibiotic disrupts the other bacteria that normally are living in the colon and preventing *C. difficile* from transforming into its active, disease-causing bacterial form. As a result, *C. difficile* transforms into its infectious form and then produces toxins (chemicals) that inflame and damage the colon. In the more severe cases, the toxins kill the tissue of the inner lining of the colon, and the tissue falls off. The tissue that falls off is mixed with white blood cells (pus) and gives the appearance of a white, membranous patch covering the inner lining of the colon. This severe form of *C. difficile* colitis is called pseudomembranous colitis .They reported that Rifampicin is a risk factor for PMC and they observed 6 cases of PMC with diarrhoea after administration of rifampicin as a treatment for active pulmonary tuberculosis[44].

Kaur et al.,(2007), conducted a study on Comparative role of antibiotic and proton pump inhibitor in Clostridium difficile infection. They studied that *Clostridium difficile* is the major aetiological agent of antibiotic associated diarrhoea and colitis. The majority of hospitalized patients infected by *C. difficile* are asymptomatic carriers who serve as silent reservoirs for continued *C. difficile* contamination of the hospital environment. *C. difficile* associated disease (CDAD) is a serious condition with mortality up to 25 per cent in frail elderly people. .They found that patient treated with PPI had a higher experimental colonisation with C. difficile[66].

Cadle et al.,(2007), conducted a study on Association of proton pump inhibitors with outcomes in Clostridium diffcile colitis.They studied that Proton pump inhibitors (PPI) inhibit the gastric acid secretion by interfering with the activity of H+ /K+ -ATPase of the parietal cells and may thus contribute to the pathogenesis of CDAD by altering the intestinal flora. PPI use was a significant risk factor for CDAD in a retrospective case control study. They found that PPI therapy was associated with an increased risk of recurrent colitis due to C. difficile[29].

Jayatilaka et al.,(2007), on Clostridium difficile infection in an urban medical center: Five-year analysis of infection rates among adult admissions and association with the use of proton pump inhibitors. They studied that the risk of CDAD due to PPI would have major public health implications. Patients are about twice as likely to develop CDAD with PPI, due to increased survival of spores. Studies from hospitals and community have examined the association between PPI use and the risk of CDAD with contradictory results .They concluded

in a five year study period found that PPI usage correlated exactly with the overall annual increased CDAD incidence and believed that the widespread prescription of PPI could be responsible[30].

Nachnani et al.,(2008), conduct a study on Proton pump inhibitors are an independent risk factor for an increased length of hospital stay in patients with Clostridium difficile infection. They stated that PPI use was a significant risk factor for CDAD in a retrospective case control study.Clinical studies carried out on inpatients at Montreal teaching hospitals comparing the risk for development of CDAD in those who received gastric acid suppressive therapy and those who did not revealed that PPI use was associated with an elevated risk of development of CDAD .They reported that PPI therapy was independent and the only risk factor associated with an increased length of hospital stay in CDAD patients. Gastric acid-suppressive drug use was also associated with an increased risk of community acquired CDAD[31].

Saxton et al.,(2009), conducted a study on Effects of exposure of Clostridium difficile.They studied that several case reports of fluoroquinolone-associated C. difficile is common .A case-control study of patients at an acute-care hospital identified ciprofloxacin use as a strong risk factor for nosocomial CDAD . The broadened anti-anaerobic spectrum of newer fluoroquinolones raises the issue of whether therapy with these agents can predispose this illness to develop in patients .They concluded that by using a 3 stage chemostat gut model showed that fluoroquinolones such as ciprofloaxacin, levofloxacin and moxifloxacin all have propensity to induce C. difficile infection[65].

Shehabi et al., (2009), conduct a study on Prevalence of Clostridium difficile-associated diarrhoea among hospitalised .they studied that Clostridium difficile, a most important nosocomial enteric pathogen, is recognized globally as responsible for antibiotic-associated diarrhea and colitis. It is associated with considerable morbidity and mortality due to widespread use of antibiotics. .they demonstrated that C. difficile is a common pathogen recovered from hospitalised patients in Jordan with symptomatic diarrhoeal illness or without diarrhoea as a result of antimicrobial usage or due to cancer chemotherapy. Thus C. difficile has emerged as the most common cause of hospital acquired diarrhoea due to broadspectrum antimicrobial use[64].

Dumsford et al.,(2009), conducted a study on Detecting hidden environmental reservoirs of Clostridium difficile. They studied that Several measures significantly reduce the incidence of CDAD. The disease can be prevented and/ or controlled when following procedures are implemented, Restriction on usage of antimicrobials, particularly for common ailments like cough, cold and sore throat. Decolonisation of asymptomatic carriers with antibiotics serves no effect. Physician treating patients who are not affected by the presence of C. difficile in their colons might cause more harm than good .They reported that in the context of CDAD outbreak, environmental contamination was common in non-isolation rooms, physician and nurse work areas and on portable equipments. Environmental contamination around asymptomatic carriers than noncarriers reflects poor toilet capacity and environmental cleaning[43].

Cohen et al.,(2010), conducted a study on Clinical practice guidelines for Clostridium difficile infection in adults. They studied that Vancomycin is also the drug of choice for the rare case of Staphylococcal enterocolitis when PMC is proven but C. difficile is undetectable by

laboratory tests. The bitter taste of vancomycin can be avoided by prescribing capsules rather than oral suspension. However, oral suspensions are preferable to achieve high concentrations in the colon more quickly in seriously ill patients. .They included that Clinical Infectious Diseases provides some information that reinforces weakly graded recommendations in treatment guidelines that currently advocate combination therapy with oral vancomycin and intravenous metronidazole in preference to vancomycin monotherapy in patients with life-threatening CDI[63].

Daryl et al.,(2013),conducted a study on the Epidemiology of *Clostridium difficile* Infection. They noted that by the 21st century, there was a marked increase in incidence and severity, occurring at a disproportionately higher frequency in older patients. They marked because older age one of the risk factor for C.D Colitis, many patients are older (< 65 yr) who are admitted and occur infections. They concluded that Enhanced surveillance methods are needed to monitor the incidence, identify populations at risk, and characterize the molecular epidemiology of strains causing CDAC[62].

Deirdre et al.,(2013), conduct a study on "Epidemiology of Clostridium difficile infection in Asia" they studied that While *Clostridium difficile* infection (CDI) has come to prominence as major epidemics have occurred in North America and Europe over the recent decade, awareness and surveillance of CDI in Asia have remained poor. Limited studies performed throughout Asia indicate that CDI is also a significant nosocomial pathogen in this region, but the true prevalence of CDI remains unknown. A lack of regulated antibiotic use in many Asian countries suggests that the prevalence of CDI may be comparatively high. . they concluded that CDI is not widely recognised in Asia so in consequence the extent of the disease

is not known. Although relatively few studies on *C. difficile* have been performed in Asia, what work has been done demonstrates that CDI is a significant cause of nosocomial disease in Asian countries. It appears that awareness is increasing and testing and surveillance are on the rise. Routine testing is required to inform on the prevalence of CDI throughout the region[61].

Khan et al.,(2014), conducted a study on "Clostridium difficile infection" where they stated that CD is the most common cause of antibiotic-associated diarrhea in hospitals and other healthcare facilities and is of significant concern because of the increasing morbidity and mortality rates as well as increased health care costs. They concluded that prompt identification of patients with symptomatic CD infection is essential as the majority of patients respond quickly to antimicrobial therapy. They recommended prevention by implementation of infection-control measures and judicious use of antimicrobial agents[58].

Rokas et al.,(2015), conducted a study on The addition of intravenous metronidazole to oral vancomycin is associated with improved mortality in critically ill patients with Clostridium difficile infection. They studied that. Vancomycin can also achieve much higher antibiotic levels in the colon than metronidazole . Metronidazole still remains first-line treatment for non-severe CDI.Recent data suggest combination therapy (IV metronidazole plus oral vancomycin) may confer an additional survival benefit in the region of 20% for critically unwell patients over vancomycin monotherapy. .They concluded that combination therapy was associated with a survival advantage in retrospective CDI cases that were (partly) matched according to illness severity[60].

Ofosu et al., (2016),conducted a study on "*Clostridium difficile* infection" where they reported that *Clostridium difficile* (*C. difficile*) infection (CDI) is the most common cause of healthcare-associated infections in US hospitals. The epidemic strain NAP1/BI/ribotype 027 accounts for outbreaks worldwide, with increasing mortality and severity. Their report stated that CDI is acquired from an endogenous source or from spores in the environment, most easily acquired during the hospital stay where the use of antimicrobials disrupts the intestinal micro flora enabling *C. difficile* to proliferate in the colon and produce toxins. They concluded that metronidazole remains the initial therapy for the majority of patients who have mild-to-moderate infection, and as the treatment of choice for the first recurrent episode of CDI. They recommended that vancomycin be the initial therapy for severe CDI and early surgical consultation will be required in the event of severe complicated CDI[59].

STUDY RATIONALE

Clostridium difficile infection has become common in healthcare settings. Clostridium difficile colitis often results after use of several drugs like antibiotics, proton pump inhibitor, immunosuppressive agent, which can have serious consequences, especially in critically ill patients. It is an infection of the colon by the bacterium, Clostridium difficile (CD). CD causes colitis by producing toxins that damage the lining of the colon. Serious complications of C. difficile colitis include dehydration, rupture of the colon, and spread of infection to the abdominal cavity, septic shock with multi organ failure. C.difficile colitis is a global health problem and in India the problem of C.difficile colitis is a major issue. The challenge is to identify patients who have risk factors for C.difficile colitis and to identify patients with infection. As CD infection is diagnosed more and more, more and more information are being generated related to risk factor, pattern of infection, outcome of those patients. In India data related to CD infection are scarce and are being generated gradually which could have implication in management of patients with CD infection. With this background my study is being proposed to evaluate patient profile with CD infection in ICU patients.

AIMS AND OBJECTIVES

The aims and objective of this study is-

- To study profile of patients admitted in ICU of a tertiary care hospital and have CD infection with special focus in previous medication, antibiotic use, immune suppression, recurrence if any and outcome.

METHODS

Study setting – The study was conducted in ICUs of AMRI Hospitals, Dhakuria unit, Kolkata. This is a tertiary care hospital with mixed medical-surgical, cardiology, neurology-neurosurgery units. The units are semi closed and both the intensive care physician and primary consultant can give opinion in management of the patients.

Study Period – the study period was from July 2018 to March 2019

Study design - the study was a prospective observational study which included all adult ICU patient population.

Study Population – Following patients were included in the study.

Inclusion criteria **-** All adult patients admitted to ICU either with features of CD colitis or had developed CD colitis were included in the study. The patients were of more than 18 years age, admitted in ICU either due to colitis or other medical condition and had nosocomial or later diagnosed having CD infection and started on an antibiotic therapy for CD treatment were included in the study.

Exclusion criteria – patients with age of less than 17 years, discharged against medical advice, withdrawn treatment and expired before microbiological diagnosis were excluded from the study.

Data collection procedure - All hospitalized ICU patients, who had proven microbiologically proven CD infection, were included in study. Only adult patients of more than 18 years age were included. Evidence of CD infection were recorded from bedside files and microbiology reports. All the data were de identified data when recorded. After a positive microbiology report the patient was included and bedside file and charts were accessed for the following variables.

Variable information–The variables for this study included demographic data like age, gender, APACHE IV score, and admission diagnoses. Comorbidities like cancer, immune suppression,

diabetes, hemodialysis, chronic liver disease and HIV infection were recorded. APACHE IV and comorbidities were recorded from the electronic database of ICU wherever applicable. History of previous admission in last one year, past CD infection and exposure to antibiotics in last three months were recorded. Current medications were also recorded. Other risk factor like days of hospitalization prior to identified CD infection, history of foreign bodies like use of rectal temperature probe andenema, presence of paralytic ileus or other pre existing colitis were recorded. Microbiologic data included stool GDH, toxin A and B, and PCR as needed. All these tests were generally recommended by the physician when patients had new diarrhea or at least 3 unformed stool in 24 hours. Drugs used for treatment of CD colitis were also noted. Treatment of CD colitis was at the discretion of the physician and no intervention was done in this area. Outcome parameters included mortality, length of stay (LOS) in ICUs, new onset of organ failure and septic shock related to CD.

All data were collected from patient file and after discussion with guide whenever there was any clinical question. Data were recordedmanually in a pre-defined excel data sheet.

Ethical Approval-

Ethical approval was taken from AMRI Hospitals Institutional Ethical Committee. Informed consent was not considered because the study was observational.

Statistical analysis

Descriptive statistics was done and has been reported as means and standard deviation, frequencies and percentages. Non parametric data has been reported as median with their interquartile ranges. All analyses was done using SPSS statistical software .

RESULTS

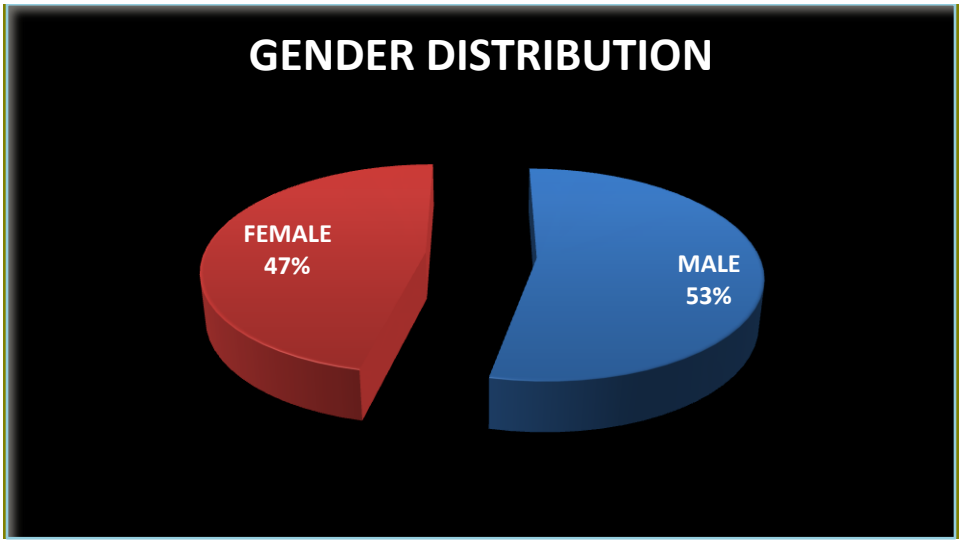
Patient characteristics

There were a total of 58 (n=58) patients with C.D infection during the study period. The mean age(±SD) of these patients was 69.6(±11.3) years; almost half of them were males (53.5%) (Figure 1). Their mean admission APACHE IV score was 67.5±28.6. Thesedemographic characteristics of the study population is described in Table 1. Gender distribution of study population in figure 1.

Table 1 Demography of study population

Variable	Results (n=58)
Age in years, mean ± SD	69.6±11.3
Sex, male n (%)	31 (53.5)
APACHE IV, mean ± SD	67.5±28.6

Figure 1 Gender distribution of study population



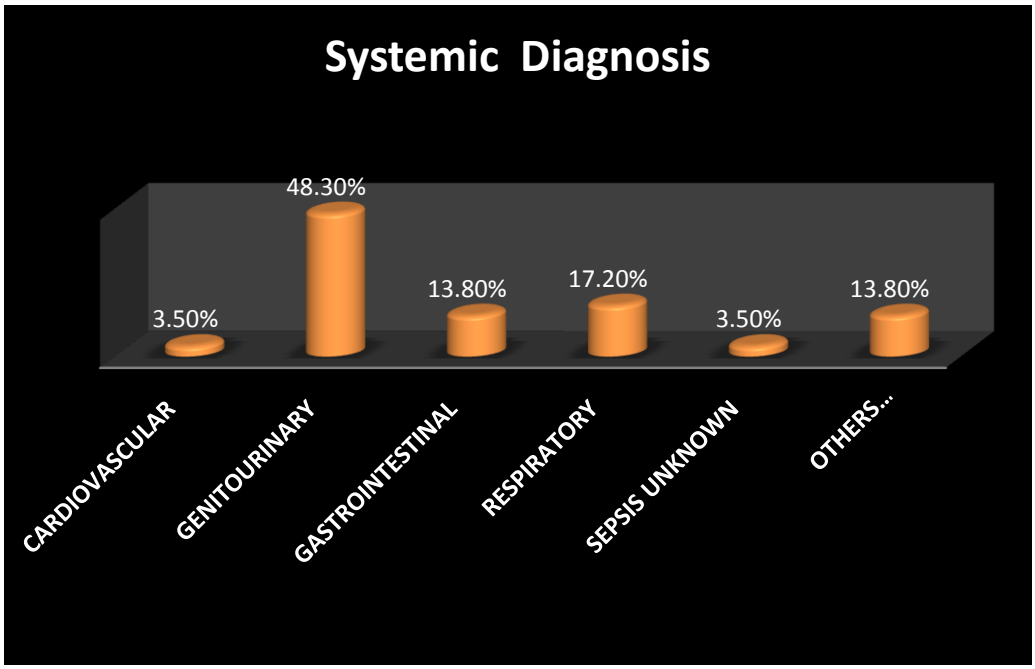
Admission diagnosis

Majority of patients (48.3%) were admitted with a genitourinary ailment (urosepsis, obstructive uropathy with sepsis etc.). 17.2% of patients had a respiratory ailment and 13.8% had gastrointestinal ailment. Other diagnosis involving this group of patients included involvement of the cardiovascular, hematological and neurological systems. 2 patients were admitted with sepsis with an unknown source. The admission diagnosis of CD patients are described in Table 2, and Figure 2.

Table 2 Admission diagnosis

Admission diagnosis, n(%)	**Results (n=58)**
Cardiovascular	2 (3.5)
Genitourinary	28 (48.3)
Gastrointestinal	8 (13.8)
Respiratory	10 (17.2)
Sepsis Unknown	2 (3.5)
Others (Metabolic, Hematological, Neurological)	8 (13.8)

Figure 2 Admission diagnosis



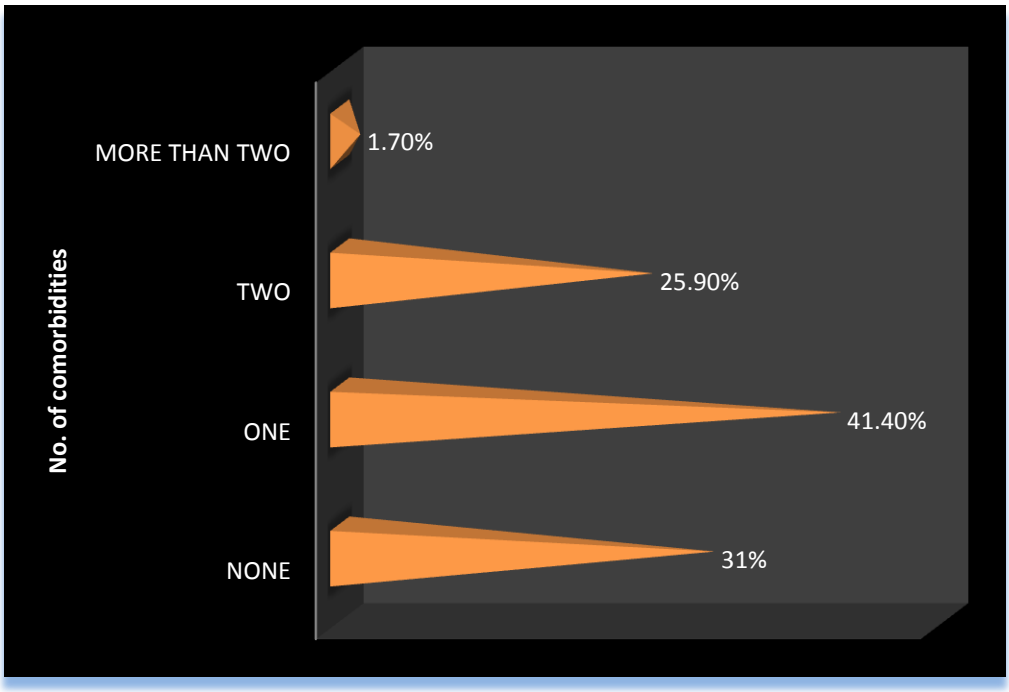
Co-morbidities

We assessed the prevalent co-morbidities in study population. 31% of patients did not have any co-morbidity, 41.4% had at least one co-morbidity and 25.9% of patients had two co-morbidities. 1 patient had more than two co morbidity (vide Table 3, Figure 3).

Table 3 Comorbidities

Comorbidities, n(%)	**Results (n=58)**
None	18 (31)
One	24 (41.4)
Two	15 (25.9)
More than two	1 (1.7)

Figure 3 Comorbidities

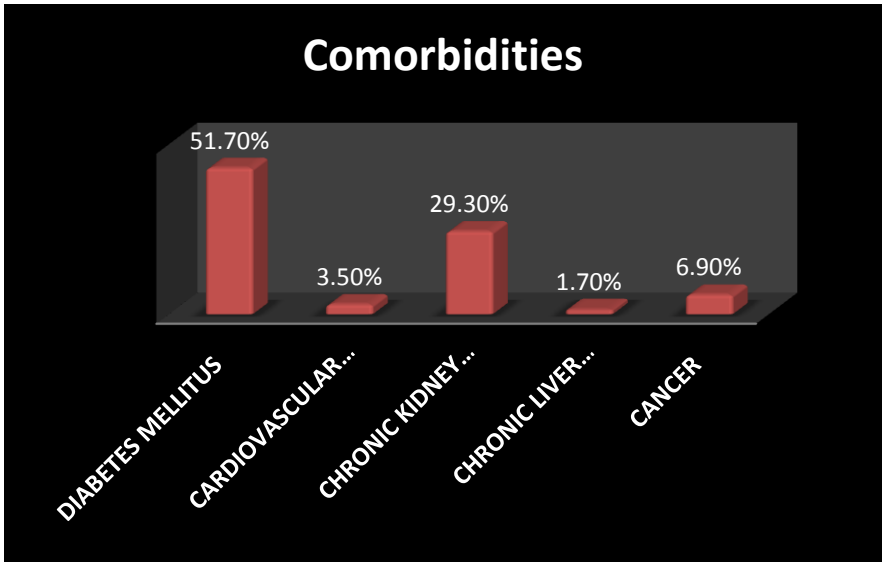


The most common co-morbidities present in our study population were diabetes mellitus, cardiovascular diseases, chronic kidney disease, chronic liver diseases and cancer. Diabetes mellitus was the commonest (51.7%) followed by chronic kidney disease (29.3%). The distribution of co-morbidities is shown in Table 4, Figure 4.

Table 4 Co-morbidities

Comorbidities, n(%)	**Results (n=58)**
Diabetes Mellitus	30 (51.7)
Cardiovascular disease	2 (3.5)
Chronic kidney disease	17 (29.3)
Chronic liver disease	1 (1.7)
Cancer	4 (6.9)

Figure 4 Co-morbidities



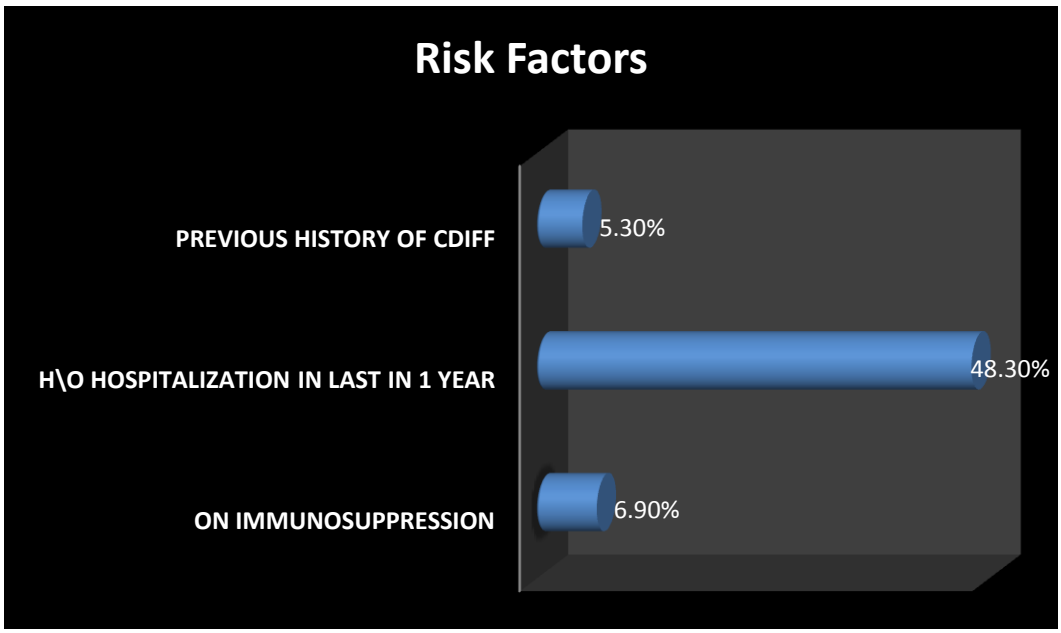
Risk factors

Risk factors for acquiring CD infection was assessed from prior history recorded on patients file. 6.9% of patients were receiving some form of immunosuppressive therapy and 48.3% of patients had a history of hospitalization in the previous year. 5.3% of patients had a prior history of CD infection. The risk factors are shown in Table 5, Figure 5.

Table 5 Risk factors

Risk factors, n(%)	Results (n=58)
On immunosuppression	4 (6.9)
H/O hospitalization in last 1 year	28 (48.3)
Previous history of CD infection	3 (5.3)

Figure 5 Risk factors



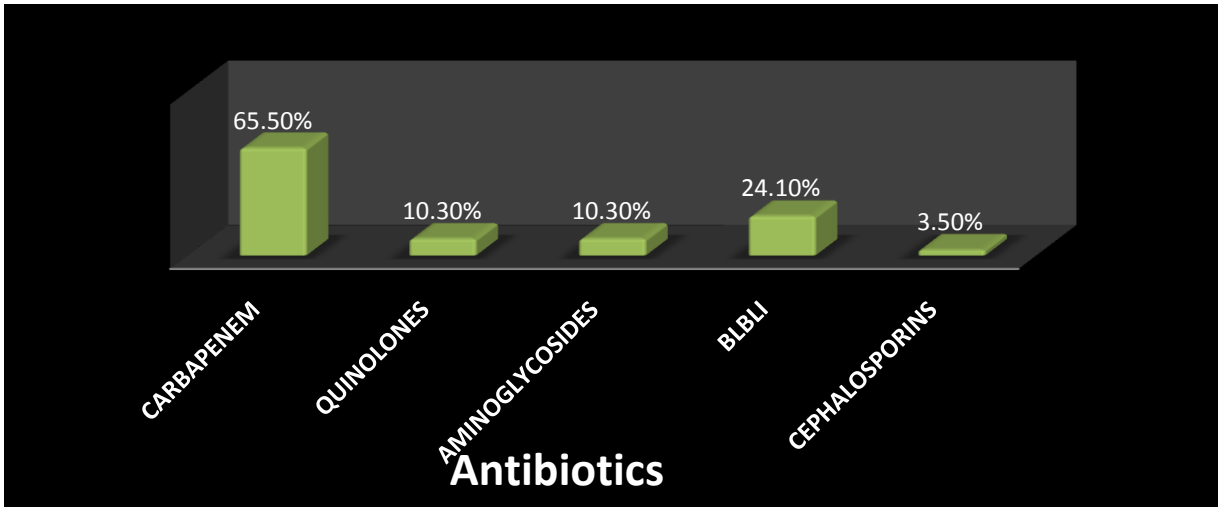
History of intake of antibiotics in recent past(Three months)

History of intake of antibiotics in recent past was assessed. 65.5% of patients had a history of carbapenem intake and 24.1% of patients had taken BLBLI at some point. Other common antibiotics that were consumed by these patients included Aminoglycosides (10.3%) and Quinolones (10.3%).Table 6 and Figure 6 shows the details of previous antibiotic intake.

Table 6 History of intake of antibiotics in recent past (Three months)

Antibiotics, n(%)	Results (n=58)
Carbapenem	38 (65.5)
Quinolones	6 (10.3)
Amino glycosides	6 (10.3)
BLBLI	14 (24.1)
Cephalosporins	2 (3.5)

Figure 6 History of intake of antibiotics in recent past (Three months)



Details on current CD infection

We examined the details of patients with CD infection. 98.3% of patients were GDH positive ,29.3% of patients had presence of A/B CD toxin and 27.6% both positive (GDH + Toxin) present in their stool The median hospital length of stay prior to CD infection was 6.5 days (IQR 3 - 11) (Table 7).

Table 7 CD diagnostic details

CD diagnostic details	Results
GDH only positive, n (%)	57 (98.3)
Toxin A/B presence, n (%)	17 (29.3)
Both positive, n (%)	16 (27.6)
Days in hospital during current hospitalization prior to CD, median (IQR)	6.5 (3 -11)

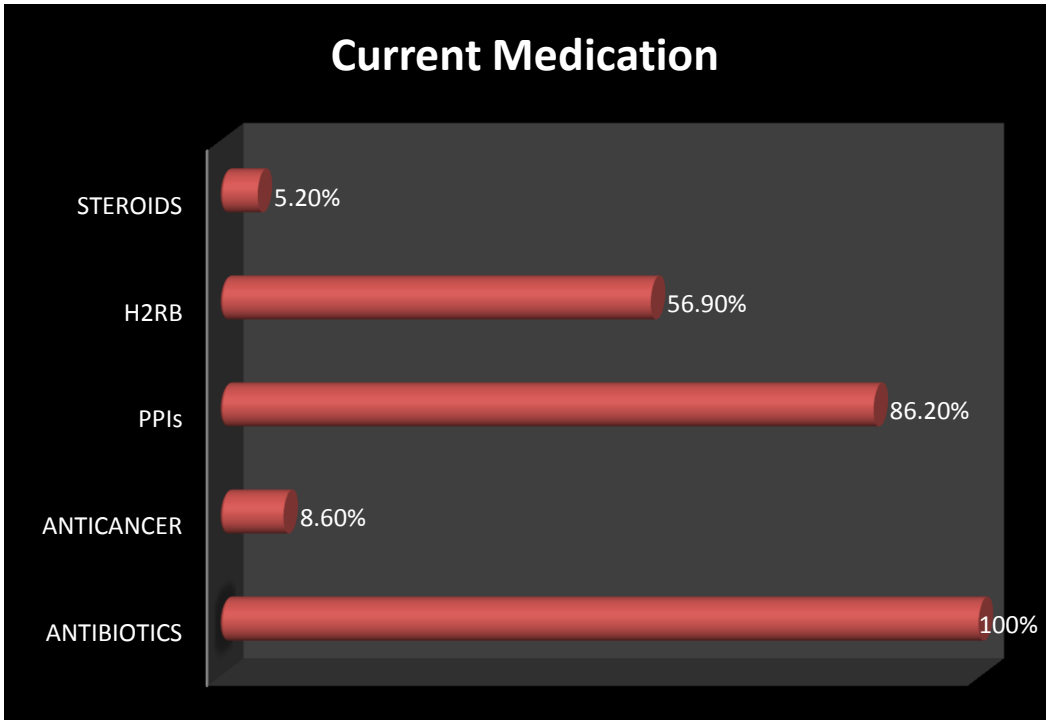
Current medications potential to predispose to CD infection

Medications taken by patient during current hospital stay with a possible potential to predispose the patient to CD infection were assessed. 100% of the study patients had received antibiotics, 86.2% of patients had received proton pump inhibitors and 56.9% had received H2 receptor blockers. The details of the current medications are shown in Table 8, Figure 7.

Table 8 Current medication

Current medications, n(%)	Results
Antibiotics	58 (100)
Anti cancer	5 (8.6)
PPIs	50 (86.2)
H2RB	33 (56.9)
Steroids	3 (5.2)

Figure 7 Current medication



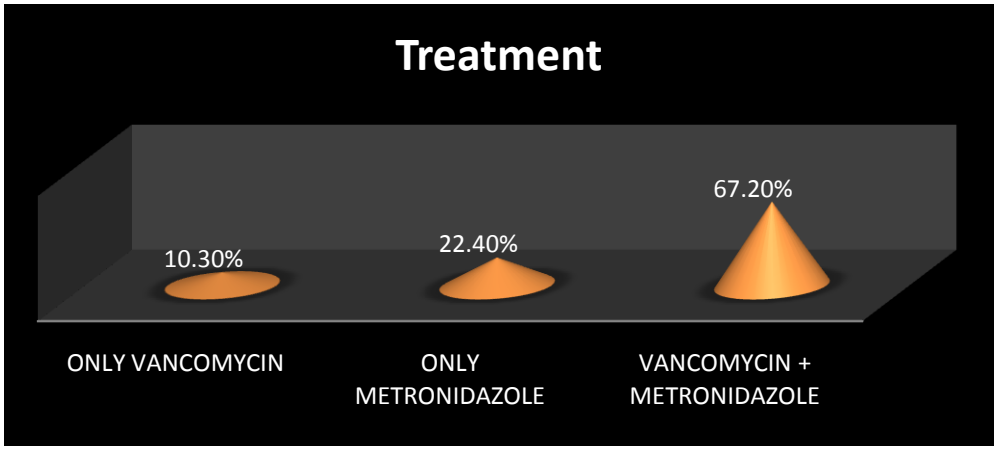
Treatment of CD colitis

67.2% of patients received both Vancomycin and Metronidazole as treatment. 22.4% patients received only Metronidazole while 10.3% of patients received only Vancomycin. The mean duration of treatment was maximum for patients in the combined Vancomycin_Metronidazole group (10.6±7.7 days). Treatment details and their duration are shown in table 9, Figure 8.

Table 9 Treatment details

Treatment received	**n (%)**	**Duration (days), mean ± SD**
Only Vancomycin	6 (10.3)	6.8±3.1
Only Metronidazole	13 (22.4)	9±4
Vancomycin + Metronidazole	39 (67.2)	10.6±7.7

Figure 8 Treatment details



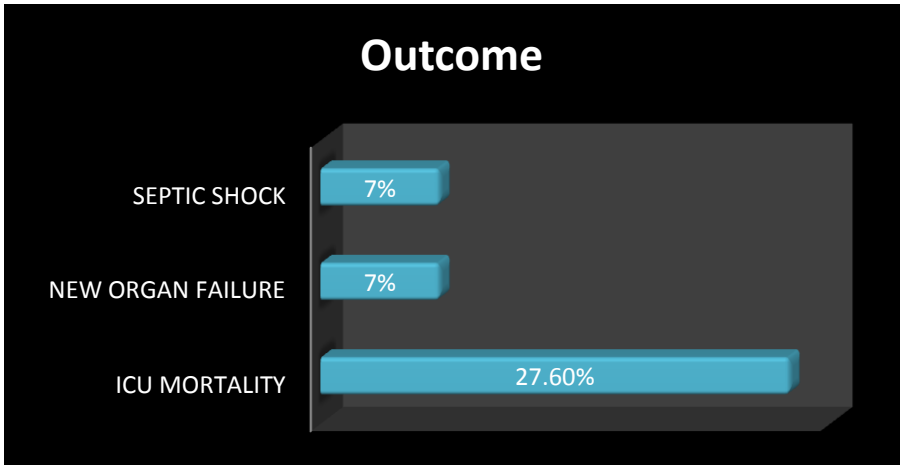
Outcome of patients with CD infection

The median length of stay for patients with CD infection was 10 days (IQR 6 -17). 27.6% of CD patients died in the ICU. New organ failure following CD infection was noted in 7% of patients. Post CD infection, 7% of patients developed septic shock. Outcome details of CD infected patients are shown in Table 10, and Figure 9.

Table 10 Outcome details of CD colitis patients

Outcome variable	Results
ICU LOS, median (IQR)	10 (6 - 17)
ICU mortality, n(%)	16 (27.6)
New organ failure, n(%)	4 (7)
Septic shock, post CDiff	4 (7)

Figure 9 Outcome details of CD colitis patients



Follow up

We followed up the stool report of CD colitis patients to assess the clearance of organism if any in stool. 3.5% of patients continued to have positive test for CD in stool and 20.7% had a negative stool report. Stool was not checked for CD in 75.9% of patients (vide Table 11, Figure 10).

Table 11 Follow up stool report

F/U stool report	**n (%)**
Positive	2 (3.5)
Negative	12 (20.7)
Not available	44 (75.9)

DISCUSSION

This study had observed the pattern of patient population in an ICU cohort who were admitted with CD colitis or acquired the same during hospital or ICU stay, predisposing factors they had, diagnostic and treatment modality and the outcome of the patients. The mean age(±SD) of the study population was 69.6(±11.3) years and almost half of them were males (53.5%). Increasing age had been identified as risk factor of CD infection in several studies which was also an important finding in our study population [73]. Apparently there was no gender distribution. Their mean admission APACHE IV score was 67.5±28.6. It represented that the infection was predominant in sicker group of patients who had a higher APACHE IV score even on admission. Mostly patients were admitted with other illness and in this cohort patients were admitted with urosepsis predominantly. Many of the co morbid conditions had a predisposition to develop CD infection in different literature[74] .Approximately one third of patients had no identified co morbidity. Diabetes mellitus was the commonest (51.7%) followed by chronic kidney disease (29.3%). In the study by Girding et al it was found that chronic renal disease and a higher baseline creatinine were risk factor for CD colitis. 6.9% of patients were receiving some form of immunosuppressive therapy and more importantly 48.3% of patients had a history of hospitalization in the previous year. Prior hospital admission was identified as risk factor for CD infection in other studies also[74]. 5.3% of patients had a prior history of CD infection which was consistent with other literatures[74]. In fact prior CD infection was identified as risk factor for recurrence in several studies. Antibiotic use is the most common risk factor for initial and recurrent CDI [75]. Although all antibiotics are associated with increased CDI risk, clindamycin, fluoroquinolones, and second generation and higher cephalosporins are associated with the highest CDI risk. In our study 65.5% of patients had a history of carbapenem intake and 24.1% of patients had taken BLBLI at some point. Other common antibiotics that were consumed by

these patients included Aminoglycosides (10.3%) and Quinolones (10.3%).Proton pump inhibitors were identified as risk factors in some studies but not confirmed in others [75,78]. Medications taken by patient during current hospital stay with a possible potential to predispose the patient to CD infection were assessed. 100% of the study patients had received antibiotics, 86.2% of patients had received proton pump inhibitors and 56.9% had received H2 receptor blockers.

Accurate diagnosis of CDI relies on a combination of clinical history and laboratory tests[79] .Enzyme immunoassays (EIAs) for toxins A and B have been a popular laboratory practice because the tests are simple to perform and results are available within 2 to 6 hours. EIAs are relatively inexpensive, easy to perform, and can provide accurate, rapid results. However, performance of EIAs can vary widely by product and can also be affected by protocol deviations, improper technique, or specimen handling. EIA sensitivity is 63% to 99%, and false-negative results can occur [80,83]. A second EIA targets the C difficile common antigen, glutamate dehydrogenase (GDH), which is secreted by C difficile into the stool [81]. GDH is an enzyme (present in most microbes) that converts glutamate to a-ketoglutarate. GDH is not specific to C difficile, and its presence does not confirm the presence of a strain of C difficile containing the PaLoc locus. However, the absence of GDH from stool is strongly predictive of the absence of C difficile, making it a potential screening assay. Nucleic acid amplification (NAAT) is the newest commercially available method for the diagnosis of CDI. Current NAATs are formatted in PCR, DNA microarray, and loop-mediated isothermal amplification methods. A result (positive or negative) is reported within 2 hours. NAAT sensitivity ranges from 84% to 96% and specificity ranges from 94% to 99% depending on the gold standard used.[85,86]. In this study, 98.3% of patients were GDH positive, 29.3% of patients had presence of A/B CD toxin present in their

stool and 27.5% patients were positive for both. One concern was that in a significant percentage of patients toxins were negative, however patients had diarrhea, or abdominal distension, fever or other new symptoms that had led to stool examination and treatment, though it did not meet the criteria of CDI as per guideline and sending a PCR was not always possible due to logistic issues. A recent study has documented that exclusive reliance on molecular tests for CDI diagnosis without tests for toxin or host response is likely to result in over diagnosis, overtreatment, and increased health care costs [87].

. Antibiotic treatment of CDI is the mainstay of therapy, and specific antibiotic treatment guideline recommendations are based on the severity of CDI disease. In this study, 67.2% of patients received both Vancomycin and Metronidazole as treatment. 22.4% patients received only Metronidazole while 10.3% of patients received only Vancomycin. The mean duration of treatment was maximum for patients in the combined Vancomycin_Metronidazole group (10.6±7.7 days). These findings were comparable to other studies [88,89].

The median length of stay for patients with CD infection was 10 days (IQR 6 -17). 27.6% of CD patients died in the ICU. New organ failure following CD infection was noted in 7% of patients. Post CD infection, 7% of patients developed septic shock. Similar information were available in other stidies. In the study by Legenza et al, mortality in patients with diarrhoea was more common in the *C. difficile*-positive group (29% vs 8%, p<0.0001). A Kaplan-Meier survival analysis (p=0.0087) for patients evaluated following a *C. difficile* test order found an all-cause mortality HR of 2.0 (95% CI 1.1 to 3.6) in patients with a *C. difficile* positive test.[90]. Overall, mean LOS for all hospitalisations reviewed was 10.2±11.0 days (median 7 days, range 0–76

days). Mean LOS for C. difficile-positive patients discharged from the hospital was significantly longer (11.3±10.5 days, median 9 days) compared with C. difficile-negative patients (8.2±8.5 days, median=6.5 days, p=0.02) [90]. 3.5% of patients in this study continued to have positive test for CD in stool and 20.7% had a negative stool report. Stool was not checked for CD in 75.9% of patients. Diarrhoea persisted, resolved or recurred in varied number of patients. These findings could not be corroborated with persistence or recurrence of infection in this study group because of lack of other supportive data.

LIMITATION

This study also had few limitation. The number of patients identified during the study period were low to find any definite correlation. This was a single center study, hence followed a particular type of diagnosis and treatment pattern. Because of observational nature of the study any additional test were not possible at any point of time. This study included patients that were admitted in ICU only.

SUMMARY and CONCLUSION

Clostridium difficile infection has become an emerging problem in healthcare settings. It is an infection of the colon by the opportunistic bacterium, Clostridium difficile (CD). CD causes colitis by producing toxins that damage the lining of the colon withserious complications. The aims of this study was to study profile of patients admitted in ICU of a tertiary care hospital and have CD infection with special focus in previous medication, antibiotic use, immune suppression, recurrence if any and outcome. It was an prospective observational cohort study in the ICU of a tertiary care hospital spreading over nine months. All adult ICU patients with CDI were included (n=58). This study had observed the pattern of patient population in an ICU cohort who were admitted with CD colitis or acquired the same during hospital or ICU stay, predisposing factors they had, diagnostic and treatment modality and the outcome of the patients. The mean age (±SD) of the study population was 69.6(±11.3) years andalmost half of them were males (53.5%). The higher mean admission APACHE IV score (67.5±28.6) represented that the infection was predominant in sicker group of patients. Mostly patients were admitted with other illness and in this cohort patients were admitted with urosepsis predominantly. Approximately one third of patients had no identified comorbidity. Diabetes mellitus was the commonest (51.7%) followed by chronic kidney disease (29.3%). 6.9% of patients were receiving some form of immunosuppressive therapy and more importantly 48.3% of patients had a history of hospitalization in the previous year. 5.3% of patients had a prior history of CD infection which was consistent with other literatures. In our study 65.5% of patients had a history of carbapenem intake and 24.1% of patients had taken BLBLI at some point. Other common antibiotics that were consumed by these patients included Aminoglycosides (10.3%) and Quinolones (10.3%).Medications taken by patient during current hospital stay with a possible potential to predispose the patient to CD infection were assessed.

100% of the study patients had received antibiotics, 86.2% of patients had received proton pump inhibitors and 56.9% had received H2 receptor blockers.In this study, 98.3% of patients were GDH positive, 29.3% of patients had presence of A/B CD toxin present in their stool and 27.5% patients were positive for both. One concern was that in a significant percentage of patients toxins were negative, however patients had diarrhea, or abdominal distension, fever or other new symptoms that had led to stool examination and treatment, and though it did not meet the criteria of CDI as per guideline and sending a PCR was not always possible due to logistic issues. A recent study has documented that exclusive reliance on molecular tests for CDI diagnosis without tests for toxin or host response is likely to result in overdiagnosis, overtreatment, and increased health care costs.Antibiotic treatment of CDI is the mainstay of therapy, and specific antibiotic treatment guideline recommendations are based on the severity of CDI disease. In this study, 67.2% of patients received both Vancomycin and Metronidazole as treatment. 22.4% patients received only Metronidazole while 10.3% of patients received only Vancomycin. The mean duration of treatment was maximum for patients in the combined Vancomycin_Metronidazole group (10.6±7.7 days). The median length of stay for patients with CD infection was 10 days (IQR 6 -17). 27.6% of CD patients died in the ICU. New organ failure following CD infection was noted in 7% of patients. Post CD infection, 7% of patients developed septic shock. Similar information were available in other stidies. 3.5% of patients in this study continued to have positive test for CD in stool and 20.7% had a negative stool report. Stool was not checked for CD in 75.9% of patients. Diarrhoea persisted, resolved or recurred in varied number of patients. These findings could not be corroborated with persistence or recurrence of infection in this study group because of lack of other supportive data.

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CONCLUSION

CDI is an emerging problem in India. This study is another supportive evidence for this statement. Older patients, history of diabetes and chronic renal disease, past history of CDI, use of antibiotics and gastric acid suppressing agents are important risk factor for CDI. Many patients had clinical symptom and GDH positive only which may lead to overtreatment. CDI patients had significant mortality, septic shock, hospital stay with their associated implication.

FUTURE RESEARCH

Future research should aim at identification of better diagnostic tool, virulent strain that may be potentially very harmful, other treatment modality as this kind of infection is difficult to cure.

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APPENDIX

Appendix 1

Data sheet

					Comorbidities									Previous antibiotics used						
ID No	**Age**	**Sex**	**APACHE IV**	**Admission diagnosis**	DM	CVD	Immunosuppression	CKD	hemodialysis	Cancer	CLD	HIV	**Hospitalization last 1 year**	Carbapenem	Quinolone	BLBLI	Cephalosporins	Amonniglycosides	others	**Previous CDiff. Infection**

Recent medications													
Antibiotics	Steroid	Anticancer	PPI	H2RB	OTHERS	**days of hospitalisation prior to Current CDiff infection**	**NJ tubes & enema & rectal probe**	**paralytic illeus or other preexisting colitis**	**Abx for Rx C diff**	**mortality**	**LOS in ICU**	**new organ failure**	**septic shock after CD**