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Clostridium difficile: Infection, diagnosis and treatment with antimicrobial drugs: A review article

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Abstract

Clostridium difficile infection (CDI) is increasing problem in healthcare, associated with high incidence, mortality, and costs in hospitalized patients. Dramatic increases in the incidence and severity of healthcare-associated *C. difficile* infection have occurred since the last decade, including elderly population, young adults, pregnant females, infants and children. *C. difficile* infections are mainly linked to the prolonged use of wide-spectrum antibiotics that disrupt the intestinal microbiota equilibrium. Toxigenic strains of *C. difficile* commonly produce two clostridial toxins, toxins A (TcdA) and B (TcdB), which are responsible for disease symptoms. Few strains of *C. difficile* may also produce another more powerful binary toxin associated with high fatality. The clinical manifestations of infection with toxin-producing strains of *C. difficile* range from symptomless carriage to mild or moderate watery-bloody diarrhea, and few percentage developed fulminant and sometimes fatal pseudomembranous colitis. Complications that have been associated with CDI include dehydration, electrolyte disturbances, toxic megacolon, bowel perforation, hypotension, renal failure, systemic inflammatory response syndrome, sepsis, and death. The most important step in treating CDI is immediately discontinuing use of offending antimicrobial drug. Both metronidazole and vancomycin are equally effective for the treatment of mild CDI, but vancomycin is superior for treating patients with severe *C. difficile* disease. Recently, fidaxomicin proved to be superior to other drugs in treatment of patients who are at high risk for CDI relapse.

Key words: *C. difficile,* Infection, Diagnosis, Antimicrobial treatment

The Genus Clostridium was first described in 1880, consists of a large number of species with a wide range of biochemical and physiological features. Clostridia are gram positive spore forming bacilli and obligatory anaerobic. Clostridium *difficile* is present in different rates as part of the indigenous human gut flora. It has also been isolated from diverse natural sources including soils, sand, and the intestinal tracts of animals. The spectrum of disease associated with *C. difficile* ranges from asymptomatic carriage to life-threatening pseudomembranous colitis. Hospitalized patients may frequently develop antibiotic associated colitis or antibiotic associated diarrhea after short or long stay in hospitals [1-2]. In recent years, *C. difficile* infection (CDI) become an increasing heath problem in elderly hospitalized patients and as community-acquired infection, and which can be associated with high incidence, mortality, and healthcare costs [3,4].

Clostridium difficile disease

The clinical and pathological features of *C. difficile* disease (CDD) indicate that it is difficult to distinguish from other similar intestinal diseases, including ulcerative colitis, chronic inflammatory bowel disease, and Crohn's disease [5]. The clinical manifestations of infection with toxin-producing strains of *C. difficile* range from symptomless carriage, to mild or moderate diarrhea, fulminant and sometimes fatal pseudomembranous colitis. *C. difficile* diarrhea may be associated with the passage of mucus or occult blood in the stool. Fever, cramping, abdominal discomfort, and a peripheral leukocytosis are also common but found in fewer than half of patients. Extra intestinal manifestations, such as arthritis or bacteremia are very rare [5].

The onset of symptoms after antibiotic treatment in adults has been ranged between 1 day to 6 weeks and longer [6], while the incubation period from antibiotic exposure to develop symptomatic CDI in children is shorter and between two to three days [7]. Almost all patients with *C. difficile* associated disease showed brown or clear watery diarrhea, but less than 50% have bloody diarrhea [5, 8-9]. The majority of patients (85%) with peudomembranous colitis appear to have mucus in their faeces, and their temperature is elevated and exceeds 38°C. Additionally, leukocytosis is a common clinical feature in the severely ill patient, and complications include dehydration, electrolyte disturbances, toxic megacolon, bowel perforation, hypotension, renal failure, systemic inflammatory response syndrome, sepsis, and death [5]. Complications are more likely to develop among neutropenic children with haematological malignancies or those treated with hematopoietic stem cell transplantation, infants with Hirschsprung's disease and patients with inflammatory bowel disease [7].

Clostridium difficile diseases are mainly linked to the use of wide-spectrum antibiotics that disrupt the intestinal microbiota equilibrium. This allows *C. difficile* to multiply and colonize the gut [10]. Intestinal colonization is an essential step in the pathogenic process of *C. difficile* and its disease depends on the loss of the commensal microbiota barrier effect following antibiotic treatment as observed in early infancy in infants [10].

According to guidelines from the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) a case definition of CDI includes the following findings : (a) the presence of diarrhea, defined as passage of 3 or more unformed stools in 24 or fewer consecutive hours1-8, (b) a stool test result positive for the presence of toxigenic *C. difficile* or its toxins, (c) a colonoscopic or histopathologic findings demonstrating pseudomembranous colitis. The same criteria should use to diagnose recurrent CDI. A history of treatment with antimicrobial or antineoplastic agents within the previous 2-month is present in the majority of patients [5].

CDI is defined as hospital-acquired if symptom onset occurred after 48 hours of admission, and

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less than 4 weeks after discharge from a healthcare facility, while CDI was defined as community-acquired if onset of symptoms occurred in the community or within 48 hours of admission to a hospital, pending no clinical symptoms of CDI observed over 12 weeks after the last discharge from a hospital [11]. Watery diarrhea is the most frequent manifestation of CDI in children due to release *C. difficile* toxins [7].

CDI may be considered if an infant with antibiotic exposure has persistent diarrhea and associated with abdominal findings that persist despite supportive care and absence of typical viral and bacterial pathogens. Additionally, if endoscopy or surgery detects pseudomembranous colitis [12].

Transmission of *C. difficile*

The organism is acquired through ingestion of spores usually transmitted from other patients through the hands of health care personnel or the environment. The spores resist the acidity of the stomach and germinate into the vegetative form in the small intestine [9]. The spores of *C. difficile* persist in a dormant state and are difficult to eradicate from the hospital environment and elsewhere [13]. The primary mode of *C. difficile* transmission is person-to-person spread through the fecal-oral route, principally within hospitalized patients. The hands of healthcare workers, are important source for transmission the organism during non-outbreak periods [5]. The major reservoirs for *C. difficile* in the hospital setting are patients with CDAD or asymptomatic carriers of *C. difficile* who heavily contaminate the hospital environment [14].

Asymptomatic carriage of *C. difficile*

Asymptomatic carriage was defined as a positive stool culture or cytotoxin test and the absence of diarrhea during hospitalization and during a 30-day period after discharge. Epidemiological Studies have indicated that the prevalence of asymptomatic colonization with *C. difficile* is ranged between 7–26% among adult inpatients in acute care facilities and is 5-7% among elderly patients in long-term care facilities [5,15]. The risk of colonization increases at a steady rate during hospitalization, suggesting accumulative daily risk of exposure to *C. difficile* spores in the healthcare setting [12]. Newborns and children in their first year of life are known to have some of the highest rates of colonization [5,7]. Infants and children are significantly more likely to carry *C. difficile* asymptomatically in the gastrointestinal (GI) tract than adults. It is estimated that 15-63% of neonates, 3-33% of infants and children younger than two years of age, and up to 8.3% of children older than two years of age are asymptomatic carriers [7]. Infants and young children rarely develop symptoms, possibly because of immature surface intestinal receptors for *C. difficile*, and because they are protected by maternal antibodies acquired transplacentally or in breast milk [7,16]. About 51% of asymptomatic patients carried toxigenic *C. difficile*, of which 37% associated with an epidemic strain [17]. Asymptomatic patient is potentially serve as a reservoir for horizontal transmission of epidemic and non-epidemic *C. difficile* strains to other patients, either by contamination of the environment or by contact with hands of medical personnel [18].

Antibiotic-associated diarrhea and pseudomembranous colitis

Generally mild to moderate diarrhea, sometimes accompanied by lower abdominal cramps is seen with *C. difficile* infection. Symptoms usually begin during or shortly after antibiotic therapy. Occasionally these may be delayed for several weeks [19]. Patients with CDI typically present with watery diarrhea and bloody stools are rare. Patients can be also presented with symptoms of colitis, fever, lower

abdominal cramps, and fecal leukocytes [19,20]. *C. difficile* toxins can be usually detected in fecal specimens, even though endoscopic and histologic features may be normal in patients with mild disease. The diarrhea resolves with discontinuation of antibiotics with 2-3 days, and the most common clinical manifestation of *C. difficile* infection is colitis without pseudomembrane formation[19]. Sometimes dehydration and a low-grade fever with a systemic polymorphonuclear leukocytosis may occur. A nonspecific diffuse or patchy erythematous colitis without pseudomembrane may be seen under sigmoidoscopy [19, 20].

Pseudomembranous colitis (PMC) is the classic manifestation of full-blown *C. difficile* colitis and is accompanied by similar, but often more severe symptoms than those observed in colitis. The classic pseudomembranes, which are raised yellow plaques ranging from 2-10 mm in diameter scattered over the colorectal mucosa. White blood cell counts of 20,000 /mm³ or greater and hypoalbuminaemia of 3.0 g/dl or lower may be observed in severely ill patients [19]. PMC lesions are nearly always limited to the colon. PMC became a commonly recognized complication of antibiotic use in the early 1950s and was primarily encountered by surgeons, who reported rates as high as 14–27% among postoperative patients. *Staphylococcus aureus* was the suspected pathogen, and vancomycin given orally became standard treatment for this condition. There are multiple other causes of PMC, including intestinal obstruction, colon cancer, leukemia, severe burns, shock, uremia, heavy metal poisoning, hemolytic-uremic syndrome, Crohn disease, shigellosis, neonatal necrotizing enterocolitis, ischemic colitis, and Hirschsprung disease. However, the vast majority of PMC cases seen since 1978 have been attributed to *C. difficile* [2,5]. Pseudomembranous colitis can only be diagnosed by direct visualization of pseudomembranes on lower gastrointestinal endoscopy; either by sigmoidoscopy or colonoscopy and by histopathologic examination. However, direct visualization using any of these techniques will detect pseudomembranes in only half of CDI cases that are diagnosed by combined clinical and laboratory tests that include both a culture positive for *C. difficile* and a positive stool cytotoxin test result. Pseudomembranous colitis can be used as a marker of severe *C. difficile* disease [2].

Fulminate colitis occurs in > 5% Patients with CDI, and patients are severely ill and associated with about 50% mortality [20]. Patients with fulminant colitis complain of severe lower and diffuse abdominal pain, diarrhea, and distension and some of them may exhibit high fever, chills and marked leukocytosis. Severe protein-losing enteropathy may result in hypoalbuminaemia. A patient with toxic megacolon has a dilated colon with signs and symptoms of severe toxicity that include fever, chills, dehydration and high white blood count [19]. The timing from onset of any CDI symptoms to fulminant colitis varies widely from weeks to just a few hours [20].

Recurrence may result from relapse of the initial infecting strain or due to reinfection with a new strain [21]. Symptomatic recurrence of CDI after successful treatment causes significant morbidity and can prove challenging to treat effectively [22]. Reported recurrence rates vary from 5% to 50% and typically are around 20%. Recurrence risk factors included older age, use of provocative antibiotics after CDI diagnosis, concomitant receipt of antacids, hospital-acquired disease, and comorbid conditions, including severe underlying illness or poor quality of life scores. CDI most commonly recurs within a week after finished treatment, but can recur after to 6–8 weeks later. About 50% of apparent relapses have been identified as new infections with a different strain [23].

Nosocomial infection by *C. difficile*

Dramatic increases in the incidence and severity of healthcare-associated *C. difficile*

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infection have occurred since 2000, particularly in patients over age 65 [3,5]. About 20% of patients with negative *C. difficile* stool cultures after admission become infected during their hospitalization. Although asymptomatic individuals are capable of shedding spores of *C. difficile* and serve as a reservoir for infection hospitalized patients [24]. New exposure and colonization by *C. difficile* are more likely causing CDI, while patients previously colonized with *C. difficile* are more likely to remain asymptomatic during their hospitalization [25]. The rate of acquisition of CDI during hospitalization is proportional to the duration of hospitalization and can be as high as 40% after 1 month [12]. Different studies showed that *C. difficile* was a major agent of nosocomial diarrhea in adults. The frequency of *C. difficile* or toxins in stool culture prescribed at least 3 days after patients' admission. *C. difficile* or toxins are recovered from 8–10% of nosocomial diarrhea [14-15]. One explanation for an increase in both the rate and the severity of *C. difficile*-associated diarrhea could be the emergence of an epidemic strain with increased virulence, antimicrobial resistance or both [26].

Risk factors of CDI

Several important risk factors for acquiring CDI have been identified and are summarized in **Table 1.** Antibacterial exposure is the most common risk for the development of CDI. Generally, every antibacterial can be associated with the development of CDI, including ironically metronidazole and vancomycin. The indigenous gut microbiota is normally protect against colonization or infection with *C. difficile*. Antibacterial can disrupt the competitive balance in the gut microbiota and promote the overgrowth of *C. difficile*. Several antibacterial classes appear to increase the risk of CDI compared to others, including clindamycin, cephalosporins and fluoroquinolones [1,27]. Recent studies showed that carbapenems place patients at a relatively higher risk for CDI, compared with other antibiotics such as first-generation cephalosporins or macrolides [20]. Cumulative antibiotic exposure via dose, use of multiple antibacterial agents, and increased days of antibiotic exposure all contribute to the risk of CDI. Alternatively, limited exposure, such as a single-dose antibacterial exposure for surgical prophylaxis also increases the risk of both *C. difficile* colonization and infection. Some investigators have hypothesized the increased incidence of CDI in young, healthy peripartum women may be a result of exposure to antibacterial prophylaxis associated with Cesarean sections [1]. The second important risk factor is mostly observed among the elderly population. This is most likely due to changes in the intestinal microbiota, an increased use of antibiotic and more frequent hospitalization [3,28]. Advanced age has repeatedly been found to be a risk factor for CDI. The risk for CDI has been thought to begin to increase at age 65. It was reported that for each year of age, the risk of health care acquired CDI increases by 2%. The increase in risk could result in part from reduced immune system function with age, particularly of the humoral immune response [9,20]. The other risk factors such as gastric acid suppression and host related factors are restricted to small group of patients [1,29].

Table 1. Patient risk factors for initial *Clostridium difficile* infection [1].

Risk for Initial episode
Antibacterial therapy
Advanced age (≥65 years)
Gastric acid suppressing agents
Cancer chemotherapy and HIV infection
Enteral feeding and gastrointestinal surgery
Healthcare exposure
Impaired immune response
Underlying chronic comorbidities

Clostridium difficile infection in infants and children

CDI has been reported in populations previously considered to be at low risk, including young adults, pregnant females, and children. Traditionally, neonates and infants were believed to be asymptomatic carriers of *C. difficile*; however, recent studies have suggested that CDI is emerging as a cause of diarrhea in infants and children [11]. Moreover, evidence suggests that a large proportion of pediatric CDI cases are community-acquired infections and that many of these infections lack the traditional risk factor of exposure to antimicrobial drugs [29]. The high rate of intestinal colonization of infants with *C. difficile* and the low rates of clinical disease appears to be due to the low capacity of the infant gut to suppress growth of *C. difficile* [31-32]. The prevalence of *C. difficile* colonization in neonates ranges from 2% - 50% with colonization often occurring within the first week of life. By approximately 2 years of age, colonization rates are similar to those in adults. However, asymptomatically colonize infants represent a potential reservoir for transmission to other family members [12]. It was initially hypothesized that infants were colonized with non-toxigenic strains of *C. difficile*, and this was the reason for the absence of disease. Potential mechanisms for disease resistant in neonates include relatively low numbers of the pathogen in the infant gut, colonization with nontoxigenic, absence of toxin receptors in the immature gut mucosa, and protective components in breast milk [16]. However, multiple studies have now demonstrated the presence of toxin-producing strains in asymptomatic neonates [32]. The American Academy of Pediatrics (AAP) Committee, recommended avoiding routine testing for *C. difficile* in children younger than 1 year of age based on the known high rates of colonization and infrequent disease. They also recommended that testing should be limited in this age group to those with risk factors, as Hirschsprung disease or other severe motility disorders or in an outbreak situation [33]. Recent data have shown that 26% of pediatric disease that was treated due to CDI occurred in infants younger than one year of age and 5% occurred in neonates [7]. Treating infants with diarrhea with antibiotics directed against *C. difficile* usually does not alter the course of the diarrhea, even if *C. difficile* is present in the stool [16]. Studies have also demonstrated that mode of delivery by vaginal, cesarean, and instrumental delivery had similar yields of *C. difficile* in stool of newborn babies. Furthermore, premature rupture of membranes (PROM), sex, and prior administration of antibiotics to the mother or the neonate had no effect on carriage rates. Longer duration of hospital stay of babies appears to increase the prevalence of the carrier state, possibly through increased exposure to *C. difficile* [16].

Pathogencity and virulence factors of *C. difficile*

The indigenous microbiota of the colon provide an important host defense by inhibiting colonization and overgrowth of *C. difficile* and other potential pathogens [34]. The acid resistance of the organism allows its spores to pass readily through the stomach, enabling germination in the small bowel on exposure to bile acids [35]. *C. difficile* produces a number of virulence factors that contribute to its virulence. These include adhesion, toxin release, hydrolytic enzyme secretion, flagella, fibronectin-binding protein, antiphagocytic capsule and host factors, and all together contribute to the pathology and feature of infection in the human host [14,36,37].

Toxigenic strains of *C. difficile* commonly produce two large clostridial toxins, toxins A (TcdA) and B (TcdB), to which disease symptoms are attributed. They are encoded by genes *tcdA* and *tcdB*. Together with three additional genes (*tcdC, tcdD,* and *tcdE*), they form the pathogenicity locus (PaLoc), of 19.6

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Table 2. Comparison of *Clostridium difficile* toxins

Characteristics	Toxin A	Toxin B
Molecular weight	308 kDa	279 kDa
Cytotoxin	+	+++
Intracellular mechanism of cytotoxity	Glycosylated Rho Proteins	Glycosylated Rho Proteins
Receptors	Present on enterocyte and other cell types	Absent on enterocyte and present on other cell types

kb, which is found only in toxinogenic strains [38]. Both toxins A and B induce mucosal injury and colitis as observed by neutrophil infiltration, which is a prominent feature of CDAD. Toxin A is an enterotoxin that causes haemorrhage and fluid secretion in the intestines of rodents whereas toxin B is a cytotoxin detectable by its cytopathic effects in tissue culture. Toxin A causes extensive damage to the epithelial lining of the intestine and acts as a cytotoxin resulting in disruption of the tight junctions of the intestinal epithelium. Toxin A initially induces cell rounding which results in detachment of the cell from the basement membrane, followed by apoptosis. After toxin A has bound to the receptor initiating the damage, toxin B joins in and gains access to the underlying tissue. The cytotoxic activity of toxin B is similar to that of toxin A, but is 1000-fold more potent than the former [19]. The prevalence rates of non-toxigenic *C. difficile* strains in healthy humans are ranged between 10-40% [15,17,38]. These strains do not produce toxins in vivo or in vitro and can colonize the gastrointestinal tract and grow normally in culture media as the toxigenic strains [15,38]. **Table 2** shows major characteristics of *Clostridium difficile* toxins.

Some strains of *C. difficile* also produce additive binary toxins CDT; an actin-specific adenine-diphosphate (ADP)-ribosyltransferase known as binary toxin CDT, which was first described in 1988 [38]. The binary toxin CDT is unrelated to the well-characterized toxins TcdA and TcdB. It belongs to the group of clostridial binary toxins, which include the iota toxin of *Clostridium perfringens* type E, the toxin of *Clostridium spiroforme*, and the C2 toxin of *Clostridium botulinum* C and D [39]. The binary toxin CDT contains the genes of *cdtA* and *cdtB*, with an organization and sequences similar to the genes of the iota toxin of *C. perfringens*: the protein sequences of CDTa and CDTb are 81 and 84% similar, respectively, to the corresponding iota toxin proteins. It was shown that only strains with changes in toxin genes *tcd*A and *tcdB* (variant strains) produce binary toxin [40]. Since the majority of strains isolated from symptomatic patients produce only TcdA / TcdB or both, and this indicates that CDT is not required for the virulence of *C. difficile*, but it may serve as an additional virulence factor [39]. Earlier studies found the binary toxin was only present in about 6% of *C. difficile* clinical isolates [5].. Binary toxin, has been detected recently in 17% to 23% of clinical strains, but its role in human disease has not been clearly defined [41].

Increased CDI incidence and severity have been attributed largely to the emergence of a new strain of *C. difficile*, designated by restriction endonuclease analysis type BI, North American pulsed-field gel electrophoresistype1 (NAP1), polymerase chain reaction (PCR) ribotype 027 (BI/NAP1/027) [8,26]. Several characteristics found in BI/NAP1/027 may contribute to its hypervirulence, including polymorphisms in an important toxin production down regulatory gene, *tcdC*; increased toxin production;

Table 3. Laboratory diagnosis of *Clostridium difficile* or its toxin in feces samples

Test	Sensitivity (%)	Specificity (%)	Advantages	Disadvantages
Stool culture & biochemical test	90-100	98-100	Allows strain typing & antibiotic susceptibility	Takes 2-5 days
EIA toxin test for both A & B	65-85	95-100	Fast (2-6 h), easy to perform, high specificity	Less sensitive than other toxin tests
Cytotoxin assay	80-90	99-100	Highly sensitive & specific	Costly tissue culture, detects only toxin B within 24-48 hours
Latex agglutination assay	58-68	80-96	Fast, inexpensive, easy to perform	Low sensitivity to detect toxin in stool
PCR for detection toxin genes	92-97	100	Excellent sensitivity and specificity compared to all tests	Done mostly in Research labs

presence of the gene encoding binary toxin (*ctdA* and *ctdB*); high-level fluoroquinolone resistance and polymorphisms in *tcdB* that could result in improved toxin binding [8]. This toxigenotype (NAP1/027) can produce 16 times more toxin A and 23 times more toxin B than control strains [42]. The NAP1 strain has entered the pediatric population at lower rates (10–19%) of toxigenic *C. difficile* isolates than reported for adults (>50%) [18,43].

Laboratory diagnosis of *C. difficile* infection

Rapid and accurate diagnosis of CDI is essential for improving outcomes of patients and the overall management of its nosocomial infection [44]. The diagnosis of CDI should be based on a combination of clinical and laboratory findings [4].

The medium cycloserine-cefoxitin fructose agar (CCFA) is the most used selective culture for recovery of *C. difficile* [5]. The second commonly used *C. difficile* moxalactam-norfloxacin agar plates (CDMN) is also highly selective for its isolation[45]. The selection of spores from feces samples by alcohol shock greatly diminishes almost all competing fecal flora and enhances both the isolation and easier recognition of *C. difficile*. The addition of bile salts, such as sodium cholate or taurocholate, to the culture medium is believed to enhance the recovery of spores by inducing the germination of spores from environmental samples or faeces after alcohol shock [46]. Optimal results require that culture plates be reduced in an anaerobic environment prior to use. The colonies are flat, yellow and have a typical odor and fluoresce with a Wood's lamp. Additionally, Gram stain shows typical morphology of gram-positive or gram-variable bacilli [5]. There are several biochemical tests, cytotoxin assay and immunological test available to confirm the identity of *C. difficile* or to detect toxin. Each test has unique characteristics as presented in **Table 3.**

Detection of ***C. difficile*** Toxins: Toxin B (cytotoxin) which is produced by toxigenic strains of *C. difficile* can be detected by different cell lines such as Vero, HEP-2 or MRC-5 monkey kidney and HeLa cell lines [5]. The first enzyme immunoassay (EIA) for detecting *C. difficile* toxin in fecal samples was developed and evaluated by Yolken *et al.*, in 1981 [47]. Commercial EIA tests have been introduced that either detect toxin A only or detect both toxins A and B. It has been the most widely used diagnostic test for CDI [8].

Detection of ***C. difficile*** Toxin by Molecular Methods: Several nucleic acid amplification tests

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(NAATs) are FDA approved for *C. difficile* testing. These assays detect conserved regions of toxin A or toxin B genes on the PaLoc of *C. difficile* [48]. NAAT utilizing either polymerase chain reaction (PCR) with excellent sensitivity (92%–97%) and specificity (100%) or loop-mediated isothermal amplification (LAMP), which appear to have similar performances is increasingly being used for diagnosis of *C. difficile* infection and healthy carriers who have diarrhea from unrelated causes [49]. (Gould *et al*., 2013). Although more expensive than traditional assays, these tests have potential for rapid and accurate diagnosis [44].

For epidemiological purpose, there are various methods currently adapted globally to type *C. difficile* isolates, including pulsed-field gel electrophoresis (PFGE), PCR ribotyping, toxinotyping based on restriction fragment length polymorphism (RFLP), restriction endonuclease analysis (REA), multilocus variablenumber tandem-repeat analysis (MLVA), and multilocus sequence typing (MLST). [50].

Treatment of *C. difficile* clinical cases with antibiotics

The most important step in treating CDI is immediately discontinuing use of the offending antimicrobial drug; and the appropriate antibiotic should be treated the patient. Additionally, providing appropriate supportive care with rehydration and electrolyte replacement as needed, and avoiding the use of antiperistaltic agents which may contribute to the development of toxic megacolon [12,51]. Once the offending antibiotic is discontinued, spontaneous resolution of CDAD symptoms will be observed in most patients within 2-4 days [5,51]. Metronidazole (Flagyl) and oral vancomycin have been the main antimicrobial agents used in the treatment of CDI. The traditional therapy for patients with moderate to severe CDI is either oral metronidazole (400 mg 8 hourly) or oral vancomycin (125mg 6 hourly) for 10-14 days. Relapse rates of *C. difficile* have been noted as high as 20% with metronidazole and 30% with vancomycin. A study has compared oral metronidazole 250 mg four times daily to oral vancomycin 250 mg four times daily, and the results suggested that oral vancomycin was superior for patients severed by severe *C. difficile*-associated disease, although the study results did not reach statistical significance [12]. Metronidazole is currently preferred in guidelines from the Centers for Disease Control and Prevention (CDC), Health Protection Agency in UK (HPA) (HPA, 2009), the Infectious Diseases Society of America (IDSA), and the Society for Healthcare Epidemiology of America (SHEA), on the basis of low cost and the concern that oral vancomycin promotes colonization with vancomycin-resistant enterococci (VRE) [5,20]. When administered orally, metronidazole is absorbed rapidly and almost completely, with only 6–15% of the drug excreted in stool. In contrast, vancomycin is poorly absorbed, and fecal concentrations after oral administration reach high levels [12]. The drug of choice for seriously ill patients is oral vancomycin (only approved by the US Food and Drug Administration (FDA) for CDI), because it has no side effect and is not absorbed by the intestine [19]. Metronidazole can be associated with severe allergic reactions as well as central nervous system toxicity. The neurotoxicity associated with metronidazole is related to cumulative exposure, and thus occurs at a higher rate following prolonged therapy, as is often used in cases where repeat *C. difficile* testing remains positive [32]. Treatment does not eradicate *C. difficile* or the toxin from the stool. Asymptomatic patients, should not be treated again if the stool test was still positive [7]. For first and second episodes of diarrhea, metronidazole is still recommended, although oral vancomycin should be considered when the patient is experiencing sepsis associated with *C. difficile* infection [20]. Newer antimicrobial agents with activity against *C. difficile* have been developed and studied in adults,

including fidaxomicin (FDA approved for treatment of CDI in adults in 2011), nitazoxanide, and rifaxamin but experience with their use in children is limited to date [32].

Antimicrobial resistance in *C. difficile*

Drug resistance of *C. difficile* towards metronidazole is still not common, while total resistance to vancomycin is not documented over the world [52]. In a recent study from Spain, a resistance rate for metronidazole (minimum inhibitory concentration 32 mg/L) was noted at 6.3%. It is expected that prolonged exposure to metronidazole can be linked to resistance. An earlier study from Spain in 2002 noted that 3% of 415 *C. difficile* isolates showed intermediate susceptibilities (MIC 4 mcg/mL-16 mcg/mL) to vancomycin [53]. Vancomycin contributes to higher selection pressure than metronidazole in developing resistance in enterococci. With the concerns of high rates of relapse, drug resistance and treatment failures, other agents are being investigated as alternative treatment strategies for CDI[52]. Furthermore, an increased rate of *C. difficile* strains show resistance to rifamycins, used for the treatment of relapsing CDI has been reported [20,52].

Several mechanisms of resistance have been identified in *C. difficile*, including acquisition of genetic elements and alterations of the antibiotic target sites. The *C. difficile* genome contains mobile genetic elements, many of them involved in antibiotic resistance. Transfer of genetic elements among *C. difficile* strains or between *C. difficile* and other bacterial species can occur through different mechanisms that facilitate their spread [54]. Antibiotic resistance plays an important role in the emergence of new *C. difficile* types. While clindamycin resistance was observed in endemic types, new epidemic types are associated with resistance to fluoroquinolones [55]. Furthermore, resistance to multiple antibiotics is a common feature observed in recently emergent epidemic isolates.

A recent Pan-European Longitudinal Surveillance has reported there was no evidence of resistance to new used fidaxomicin, and reduced susceptibility to metronidazole and vancomycin was also scarce. Rifampicin, moxifloxacin, and clindamycin resistance accounted to 13%, 40%, and 50% of total isolates, respectively and were associated with multiple ribotypes [56]. The rapid identification of new phenotypic and genotypic traits, the implementation of effective antimicrobial stewardship and infection control programs, use of alternative drugs are all important factors to prevent and control the spread of resistance and to ensure a successful therapy for CDI.

Biotherapy of *C. difficile* clinical cases

Probiotics such as lactobacillus species and *Saccharomyces boulardii*, have shown efficacy in reducing the incidence of simple antibiotic-associated diarrhea, but their efficacy in preventing *C. difficile* infection is not consistent, however, re-establishment of a healthy intestinal flora is the aim of probiotics [20,57]. There are limited data to support this treatment approach and there is a potential risk of bloodstream infection. Until recently, no single study had shown clearly probiotics to be effective in the prevention of CDI [5].

Clostridiun difficile infection in Arab countries

There are few epidemiologic data published on *C. difficile* in Arab countries over the last 15 years according the search in 2 most medical famous indexing web sites (Pub Med & Scopus). In Jordan,

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Shehabi *et al*. [9], performed a study at the Jordan University Hospital in 2000 and found a prevalence rate of 9.7% *C. difficile* isolates or its toxin in patient stools of all ages with diarrhea by using culture and enzyme immunoassay for the detection of *C. difficile* toxin A. A study done in Kuwait by Rotimi *et al*. in 2002 [58], reported that the prevalence of hospital acquired *C. difficile* infection/colonization was less than 10%. A second study conducted in Kuwait focused on the PCR ribotyping of environmental and ICU clinical strains. A total of 32 different ribotypes were detected among the clinical isolates, and the predominant toxigenic ribotypes detected were types 097 and 078, which are different from the findings obtained in North America and Europe, which exhibited a dominance of the 027 ribotype [59].

Nasereddin *et al*. [15], reported an increase in CDI rates observed at the Jordan University Hospital after abot 8 years of the first study done in the same hospital. The study showed a prevalence of toxigenic *C. difficile* isolates was 13.7% among adult hospitalized patients, and 73% of the *C. difficile* isolates carried tcdA and/or tcdB toxin genes as demonstrated by PCR and in association with diarrhea, and all *C. difficile* isolates were negative for binary toxin production. A recent multicenter study held in Jordan in 2015 by Wadi *et al*. [3], detected a prevalence rate of 92.4% among hospitalized old patients with *C. difficile* toxin-positive diarrhea-stools using a rapid test, which detects fecal *C. difficile* toxins A and B. The adults and older age groups accounted for the majority of all positive cases. In Saudi Arabia, a study reported a prevalence rate of 4.6% of *C. difficile* among Saudi patients. Stool analysis for *C. difficile* toxins A and B was carried out by an enzyme-linked immunosorbent assay [60]. In Qatar, Al-Thani *et al*. [61], recently reported a prevalence rate of CDI (7.9%) amongst their 1,532 hospitalized patients, and their study used glutamate dehydrogenase (GDH) lateral flow assay and toxins A and B Enzyme Immunoassay (EIA) fordetection of *C. difficile*. A recent Lebanese study demonstrated that 30 out 88 (65.2%) of stool samples examined of patients admitted to different hospital units at the American University Medical Center in Beirut, were positive by culture for *C. difficile* or its toxins (*tcdA*/*tcdB*) or both genes. Their results of toxinotyping showed that 2 isolates belonged to toxinotype 0, 4 to toxinotype XI, 2 to toxinotype XII, 1 to toxinotype XVI, 1(A+B-) and twenty (A-B+) designated as toxinotype 0-like [62]. The frequency, demography, clinical features and outcome of nosocomial CDAD in children admitted to Assiut University Children's Hospital, Egypt, was investigated by a descriptive cross-sectional study. Out of 72 examined children, 17 (23·6%) were diagnosed with CDAD using culture for *C. difficile* and direct toxin detection from stool samples by enzyme immuno-assay. Those aged ≤12 months were the most commonly affected (eight, 47%). The main cause of admission was respiratory disorders (47% of cases) [63].

Lastly, a new not published yet study from Jordan (personal communication, 2016), indicated that the overall *C. difficile* colonization rate among Jordanian infants aged less than one year was (37/287; 12.9%). Both toxigenic (54%) and non-toxigenic *C. difficile* strains (46%) were detected. Additionally only one isolate (2.7%) was positive for binary toxin. All isolates of *C. difficile* were susceptible to vancomycin and metronidazole, while high resistance rate to ciprofloxacin and low resistance rate to erythromycin were detected among the isolates. The results of fluoroquinolone resistance determining-genes indicated that (40.5%) of the isolates carried both *gyrA* and *gyrB*.

The presence of toxigenic *C. difficile* in stools of infants without diarrhea, suggests that they were asymptomatic carriers of toxigenic strains; thus infants may be a potential reservoir to spread infection with these trains to other close contact.

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