Gut Pathogens

REVIEW

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Risk factors and outcomes of *Clostridioides difficile* infection in patients with colorectal cancer: critical perspective in management

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Abstract

Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide, causing a serious threat to global health and social burden. *Clostridioides difficile* infection (CDI) is one of the most important nosocomial infections and has a higher incidence in cancerous population compared with non-cancerous cases. Different risk factors, including gut microbiota dysbiosis, extensive surgery, chemotherapy, prolonged hospitalization, and antimicrobial therapy, compromise host defenses against CDI and contribute to cancer patients' susceptibility to this infection. The emergence of CDI in patients with CRC creates conditions for therapy escalation and prolonged hospitalization, highlighting the need for correct and effective CDI management in these patients. Here, common risk factors associated with CDI in patients with CRC are discussed. In addition, different available techniques for the prevention, detection, and treatment of CDI with the lowest impact on gut microbiota diversity are summarized. This review aims to improve the understanding of the interplay between CDI and CRC and provide new insights into restoring and maintaining gut microbiota balance during CDI management in patients with CRC.

Keywords Colorectal cancer, *Clostridioides difficile* infection, Diagnosis, Treatment, Gut microbiota

Background

Colorectal cancer (CRC) is the third most prevalent malignancy and the second deadliest, with 1.8 million new cases and 881,000 deaths worldwide in 2018 [1]. Importantly, CRC was identified as the cause of 10% of new cancer cases and cancer-related deaths globally in 2020 [2]. CRC is caused by genetic mutations and epigenetic alterations in colonic cells, leading to the conversion of epithelial cells into adenocarcinomas [2, 3]. In this regard, somatic/acquired or inherited genetic mutations and environmental factors, including dietary food regimes, physical inactivity, and smoking, are risk factors related to the prevalence of CRC [4, 5]. Recently, dysbiotic microbiota has been identified as a key factor in the genesis of cancer progression [6], which is typically marked by a decreased level of beneficial microbes and an increased level of enteric pathogens [7].

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Surgery and chemotherapy are the main conventional approaches for CRC treatment [8]. Surgery is the primary treatment approach for early-stage CRC tumors, whereas chemotherapy regimens are administered for advanced and metastatic stages [9]. Although the combination of these approaches has significantly improved patient survival, their long-term effectiveness is limited [8]. In addition, these methods may lead to different gastrointestinal [GI] complications, such as mucositis, necrotizing and ischemic colitis, and severe diarrhea [10]. An imbalance in gut microbiota can also occur due to the use of these therapies [11, 12].

Changes in gut microbiota composition create an environment conducive to tumorigenesis and tumor progression through the carcinogenic activities of certain bacterial species, production and alteration of gut microbiota metabolites, and stimulation of immune system responses [13, 14]. An imbalanced microbiota may also contribute to the development of GI infectious diseases in patients [15, 16].

Recent studies have demonstrated that dysbiosis is a precursor to *Clostridioides difficile* infection (CDI) [17–20]. This infection is known as the most important hospital-acquired infection, and its prevalence has recently increased in the USA, with an overall incidence rate of approximately 121.2 cases per 100,000 according to the Centers for Disease Control and Prevention (CDC)'s surveillance report [21, 22]. The relationship between CRC and CDI has been investigated in several studies, demonstrating higher CDI rates and more severe outcomes in CRC patients compared with non-cancer patients [23–26]. In addition, individuals with CRC frequently experience surgery, chemotherapy, antimicrobial treatment, and extended hospital stays, which these factors predispose to gut dysbiosis and CDI [27]. However, most available data on CDI rates are collected from non-cancer individuals, and the precise prevalence and risk factors of CDI in patients with CRC are not fully understood. In addition, studies on the incidence of *C. difficile* in patients with cancer are mostly limited by insufficient sample-size, and there are no comprehensive epidemiological studies on CDI outcomes in cancer populations [27]. Previous studies have demonstrated that CDI may cause complications in cancer patients, such as extended hospitalization time, severe diarrhea, and altered response to therapy [28, 29]. Accordingly, understanding the interplay between CRC and CDI is important for enhancing patient care, improving clinical decision-making, and providing more effective treatment management during infectious events. This review discusses the risk factors for CDI development in patients with CRC and introduces promising potential strategies for preventing, detecting, and treating this infection in these patients.

C. difficile infection (CDI)

CDI is a global infectious disease caused by a gram-positive spore-forming anaerobic bacillus, which is transmitted via the oral-fecal route [20]. CDI has been categorized as endogenous or exogenous: endogenous CDI originated via *C. difficile* strains already carried by patients, whereas the development of exogenous infection is associated with *C. difficile* acquisition from infected individuals, contaminated environments, and healthcare workers [30]. *C. difficile* spores have a high tolerance to unfordable conditions and even the acidity of the stomach. The ingestion of spores can lead to CDI infection in individuals with a disrupted or altered gut microbiota and/or immunosuppressed [31].

The CDI spectrum of symptoms includes mild-to-moderate diarrhea, and more severe manifestations, such as pseudomembranous colitis (PMC) and toxic megacolon, which may be life-threatening for patients, especially elderly individuals [20, 32]. The pathogenesis of *C. difficile* is associated with the production of several virulence factors, such as toxins and surface proteins [33]. The toxin A (TcdA) and toxin B (TcdB) are key virulence factors of *C. difficile*. These toxins belong to the family of clostridial glycosylating toxins and are encoded within the pathogenicity locus (PaLoc) [32, 33]. In addition, hypervirulent strains can produce a binary toxin or *C. difficile* transferase (CDT), which facilitates CDI development in patients [34]. These toxins can be internalized into epithelial cells, resulting in cell death and loss of intestinal barrier function [32]. The pathogenic effects of toxins may be further boosted by triggering host immune responses, such as the induction of acute inflammation and neutrophil infiltration, leading to further damage to epithelia cells [33].

The main risk factors for CDI development are antibiotic therapy, long-term hospitalization, advanced age (> 65 years), chemotherapy, use of gastric acid suppressors, such as proton pump inhibitors (PPIs), immunosuppressive therapy, renal insufficiency, or prior gastrointestinal surgery [35, 36]. Most of these risk factors lead to dysbiosis in the gut microbiota composition [7, 12, 20, 37, 38].

Although different novel therapies have been introduced for treating CDI, including fecal microbiota transplantation (FMT), antibody therapy, and phage therapy, the most common method for treating this infection for many years has been antibiotic therapy, including metronidazole, vancomycin, and fidaxomicin [39–41]. The updated treatment guidelines by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) in 2017 recommend metronidazole only for patients with a first episode of non-severe CDI where vancomycin or fidaxomicin are unavailable. In addition, although fidaxomicin and

vancomycin have been introduced as acceptable therapy for CDI, fidaxomicin has a preferential choice over vancomycin for initial CDI [42]. Notably, antibiotic therapy may disrupt the composition of the gut microbiota and provide a suitable environment for *C. difficile* recolonization [20]. There are shreds of evidence demonstrating that antimicrobial treatment regimens increase the risk of relapse up to eight to ten times, and the risk remains three times higher for four weeks after discontinuing therapy [20, 43], leading to an increase in the rate of recurrence in patients with CDI.

Risk factors for the development of *C. difficile* infection in colorectal cancer patients

Several studies have indicated a potential risk of CDI development in CRC patients [23, 26, 44]. CRC patients are at a higher risk of CDI due to combination of factors, including dysbiotic gut microbiota, colorectal surgery, prolonged exposure to chemotherapeutic regimens, antibiotic therapy, aging, prolonged hospitalization, and altered levels of bile acids in the intestine [45]. All of these factors contribute to dysregulating the mucosal immune responses in CRC patients and to decreasing diversity in the gut microbiota composition, favoring possible colonization by pathogenic or opportunistic bacteria, and creating an environment conducive to CDI [27, 46].

Gut microbiota dysbiosis

A healthy gut microbiome includes many bacterial species that help maintain gut homeostasis (Fig. 1A). Altered intestinal microbiota has been identified as a critical factor CRC development and progression (Fig. 1B) [47]. There is growing evidence about the dysbiotic state of the microbiota in patients with CRC, specified with an increase in the abundance of Proteobacteria and a decrease in the abundance of Firmicutes, Bacteroidetes, and Actinobacteria. Particularly, an increased abundance of different bacterial species, such as *Fusobacterium nucleatum*, *Escherichia coli*, *Enterococcus faecalis*, enterotoxigenic *Bacteroides fragilis*, *Streptococcus bovis*, *Gemella* species, *Parvimonas*, *Peptostreptococcus anaerobius*, *Anaerobutyricum hallii*, *C. difficile*, and *Heliobacter pylori* has been reported in the gut microbiota of CRC patients [48, 49]. In addition, there is evidence that supports the carcinogenic role of some of these bacteria, such as enterotoxigenic *B. fragilis*, *E. coli* producing colibactin, *F. nucleatum,* and toxigenic *C. difficile* [50–54]. Conversely, decreased abundance of different bacterial species, such as *Bifidobacterium*, *Roseburia*, and *Clostridium butyricum*, has also been reported in patients with CRC [55]. Furthermore, changes in microbial metabolites, such as bile salt hydrolases (BSH), short-chain fatty acids (SCFAs), amino acids, and lipopolysaccharides, can affect CRC progression and impact on diarrhea development in CRC patients [56].

Notably, gut microbiota dysbiotic state and dysregulation of microbial metabolites in CRC patients facilitate *C. difficile* colonization and provide an environment conducive to CDI occurrence. In fact, alteration in gut microbiota composition, including an increase in the abundance of Proteobacteria (especially *Enterobacteriaceae*] and a decrease in the abundance of Firmicutes (especially *Lachnospiraceae* and *Ruminococcaceae*), Bacteroidetes (especially *Bacteroides*), and Actinobacteria (especially *Bifidobacterium*), are risk factors for *C. difficile* colonization [57–59], which can also be found in the microbiota composition of patients with CRC. Therefore, dysbiosis in the gut microbiota composition is a crucial factor for CDI development in patients with CRC.

Colorectal surgery

Colorectal surgery is considered the primary treatment for CRC. It has been documented that colectomy and gastric or esophageal surgery, as well as ileostomy closure after rectal cancer, may increase the risk of CDI in hospitalized patients [37, 60]. Previous studies have demonstrated that the rate of CDI in patients undergoing colorectal surgery is nearly three times greater than in non-surgical patients [38, 61, 62], and CDI might be more common in CRC patients undergoing colorectal resection [24, 38]. In addition, the intestinal microbiota composition significantly differs between pre- and post-surgery CRC patients. In two recent studies, a decreased abundance of Bacteroidetes and Firmicutes and an increased abundance of Proteobacteria and Fusobacteriota have been observed in post-operative compared to pre-operative CRC patients [12, 63, 64]. At the genus level, an increased abundance of *Escherichia*, *Klebsiella*, *Shigella,* and *Faecalibacterium* has been observed in CRC patients undergoing radical surgery compared to pre-operative patients, as well as a decreased abundance of *Streptococcus* and *Lactobacillus* [12, 65]. These observations suggest that gut dysbiosis induced by surgery may facilitate gut colonization by *C. difficile*; therefore, managing CDI in post-operative CRC patients is paramount.

Consumption of chemotherapy drugs

Different chemotherapeutic drugs are recommended for the treatment of CRC. The three major regimens of chemotherapy for advanced CRC include: the FOLFOX regimen (folinic acid in combination with 5-fluorouracil (5-Fu), oxaliplatin), the FOLFIRI regimen (5-Fu in combination with irinotecan), and the CapeOx regimen (capecitabine in combination with oxaliplatin) [11]. The use of chemotherapy can cause various side effects in patients. It has been observed that 40% of patients

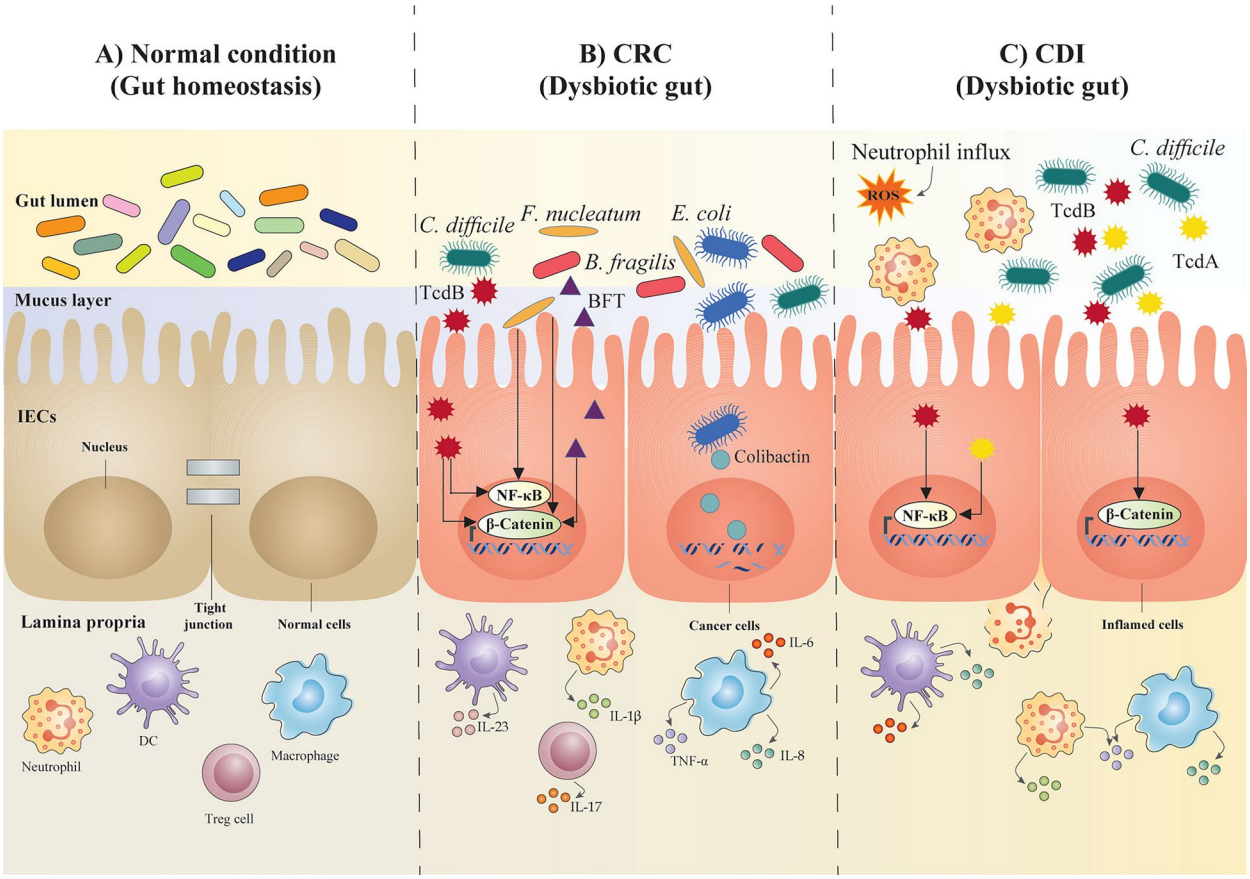

Fig. 1 Schematic representation of the composition of the gut microbiota in healthy and disease states. **A** A healthy gut microbiota is composed by a high taxonomic diversity of bacterial species, helping the maintenance of gut homeostasis. **B** In CRC patients, a dysbiotic gut is caused by various factors, such as antibiotic therapy and chemotherapy, resulting in the enrichment of pathobionts, such as *F. nucleatum*, enterotoxigenic *B. fragilis*, *C. difficile*, and *E. coli*. These bacteria induce DNA damage and activate different antigen-presenting cells (APCs), including macrophages, DCs, neutrophils, and T cells, which produce pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-23, and TNF-α), and signaling pathways related to inflammation and cancer progression, such as the NF-κB and Wnt/β-catenin signaling pathway. Activation of these signaling pathways and DNA damage contribute to tumorigenesis. **C** Gut microbiota dysbiosis increases the susceptibility to *C. difficile*. The adherence of *C. difficile* to intestinal epithelium activates APCs increases levels of cytokines, such asIL-6, IL-8, and TNFα, and contributes to neutrophil influx, which determine pseudomembrane formation, that characterizes *C. difficile* colitis. In addition, internalization of toxin TcdA and toxin TcdB activate signaling pathways related to inflammation and the release of inflammatory cytokines. TcdB may also play a role in tumorigenesis through activating Wnt/β-catenin signaling. APCs antigen-presenting cells; BFT *Bacteroides fragilis* toxin; *B. fragilis*, *Bacteroides fragilis*; *C. difficile*, *Clostridioides difficile*; CDI, *Clostridioides difficile* infection; CRC, colorectal cancer; *DCs* dendritic cells, *IL* interleukin; *E. coli*, *Escherichia coli*; *F. nucleatum*, *Fusobacterium nucleatum*, *NF-κB* nuclear factor κB, *TcdA* toxin A, *TcdB*, toxin B, *TNF-α* tumor necrosis factor alpha, *Wnt* wingless-related integration site

undergoing standard-dose chemotherapy and 60–100% of patients undergoing high-dose chemotherapy experience malnutrition caused by intestinal mucositis, nausea, abdominal pain, vomiting, and diarrhea [66, 67]. In addition, chemotherapeutic regimens, such as FOLFOX, antimetabolites, alkylating agents, and platinum complexes, can activate toll-like receptor (TLR) signaling pathways and subsequently elevate inflammatory cytokines and reduce mucosal regeneration [7]. Alteration of the gut microbiota is a crucial side effect of chemotherapy [68]. This method can directly or indirectly exert high-impact side effects on the microbiota composition, such as malnutrition, hepatotoxicity, gastrointestinal toxicity, and mucositis [68, 69]. Clinical studies have demonstrated that the intestinal microbiota shift may be peculiar for the different chemotherapy regimens used, although it is generally characterized by a decrease in the Firmicutes (e.g., *Ruminococcus, Blautia* and *Lachnospira*) and Actinobacteria (e.g., *Bifidobacterium*) abundance, and an increased abundance in Proteobacteria (e.g., *Staphylococcus*, *Enterobacter* and *Escherichia*) [11, 68, 70, 71]. Pathological changes in the gut microbiota, such as intestinal mucositis and severe diarrhea, have also been reported in patients receiving systemic chemotherapy [11, 72, 73].

Although antibiotic therapy is commonly cited as the primary risk factor for CDI, chemotherapy can also contribute to CDI development in the absence of antibiotics [74, 75]. Several studies have demonstrated that side effects induced by chemotherapy have adverse consequences and reduce colonization resistance against *C. difficile* and other enteric pathogens in cancer patients [76–78]. Altered gut microbiota in patients who have undergone chemotherapy is an important factor associated with an increased incidence of CDI in patients with CRC [79]. The low diversity of the microbiota composition in patients under chemotherapy provides a conductive environment for *C. difficile* colonization and CDI development [7, 79]. In addition, chemotherapeutic agents, such as 5-Fu and methotrexate, can induce PMC formation, which is a characteristic manifestation of CDI development [80, 81]. Kamthan et al. demonstrated that the incidence of *C. difficile*-associated diarrhea (CDAD) in patients receiving chemotherapy without any antimicrobial therapy was approximately 5.7% [82]. Zheng et al. found that patients with metastatic lymph nodes, receiving adjuvant chemotherapy, experienced higher *C. difficile* colonization rates (22.3%) compared with patients with not metastatic lymph nodes that not receiving adjuvant chemotherapy (10.8%), supporting that chemotherapy may increase the rate of CDI in patients [83]. Notably, potent anti-diarrheal agents, such as octreotide, are not recommended for patients experiencing chemotherapy-induced diarrhea with fever or bloody stool due to concerns about infectious colitis. In fact, these drugs may increase the cytotoxic effects of the *C. difficile* toxin, leading to clinical deterioration [84]. Furthermore, it has been documented that the chemotherapeutic paclitaxel may induce intestinal inflammation and exert antimicrobial activity on enteric bacteria, providing a niche for *C. difficile* overgrowth [84]. In addition, prior antibiotic use may exacerbate paclitaxel-induced diarrhea [84], promoting dysbiosis and boosting the destructive effect of paclitaxel, which may induce severe CDI. However, the importance of paclitaxel as a risk factor for *C. difficile*-associated colitis remains unclear. Further research is required to clarify the interplay between the types of chemotherapeutic drugs used and the incidence of CDI.

Antibiotic therapy

Antibiotic therapy is a significant risk factor for CDI development, especially with broad-spectrum antibiotics such as cephalosporin, clindamycin, fluoroquinolones, and penicillin. Broad-spectrum antibiotics alter the gut microbiome, dysregulate bile acid metabolism and bacterial metabolites, and provide ideal conditions for *C. difficile* germination and outgrowth [18, 85, 86]. In addition, recent insights have indicated that the excessive use of broad-spectrum antibiotics can lead to the emergence of antibiotic-resistant bacterial strains, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, due to horizontal gene transfer by mobile genetic elements like plasmids and transposons [87–91]. The emergence of these strains requires the use of more potent antibiotics, which in turn leads to a heightened risk for CDI. In addition, the increased abundance of these bacteria may boost dysbiosis [92, 93] and consequently facilitate *C. difficile* colonization.

Antibiotics may be administered to patients with CRC for different purposes, such as managing sepsis or surgical infections and enhancing chemotherapy results [94, 95]. The use of antibiotics, such as prophylaxis, in patients with CRC who have undergone surgery for tumor removal is considered a standard of care for reducing surgical site infection and overall mortality and improving surgical outcomes [96]. Nonetheless, a higher risk of CDI following prolonged consumption of antibiotic prophylaxis has been reported [97]. Notably, the use of single-dose metronidazole has been suggested as an effective treatment for reducing post-operative diarrhea and CDI in patients who underwent ileostomy reversal surgery [98]. However, preclinical and clinical studies have demonstrated that both metronidazole and vancomycin affect the microbiome, bile acid metabolism, and host physiology [86, 99, 100].

Several studies have suggested that combining antibiotics with chemotherapy could be a potential therapeutic option to improve treatment efficacy. Imai et al. demonstrated that combining antibiotics with oxaliplatin enhanced the treatment effectiveness in advanced CRC patients [94]. Oxaliplatin treatment does not affect the diversity of the gut microbiota but causes a significant increase in the gram-negative microbiota at the genus level [101].

The administration of antibiotics like moxifloxacin or azithromycin in conjunction with chemotherapy could increase the efficacy of treatment [102, 103] but can negatively impact the gut microbiota of cancer patients and may cause intestinal overgrowth of pathogenic strains, including toxigenic *C. difficile* strains [104, 105]. In addition, the use of both chemotherapeutic drugs and antibiotics may contribute to an increase in drug-resistant strains of the microbiota, raising concerns about a higher infection rate [106]. Nonetheless, prophylaxis is still recommended for post-operative cancer patients due to its effectiveness in clinical studies [107].

Elderly population and hospitalization

Current epidemiological research has shown that age > 50 years is a significant risk factor for CRC [108]. Furthermore, elderly individuals (> 65 years) have an

increased risk of acquiring *C. difficile*, probably because of more usage of PPIs, antibiotics, hospitalization, and exposure to healthcare-associated infections [109, 110]. In addition, these patients may experience a higher risk of morbidity and mortality due to CDI compared with younger individuals [109, 111].

Age-related alterations in physiology and immunity could be involved in the alteration of the gut microbiota diversity, which is strongly associated with CDI [112, 113]. Several studies have reported changes in the gut microbiota related to age [114–116]. In particular, elderly hospitalized patients often exhibit decreased microbial diversity in their intestinal microflora, characterized by a decrease in the abundance of *Bacteroidaceae*, *Lachnospiraceae*, *Ruminococcacae*, *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium*, and an increase in the abundance of *Enterococcaceae* and *Enterobacteriaceae* [117, 118], which may compromise the gut environment for *C. difficile* growth. Interestingly, Yoem et al. have demonstrated that an age ≥ 60 represents a risk factor for *C. difficile*-associated colitis among post-operative CRC patients [62], highlighting the importance of an appropriate CDI management in elderly patients with CRC.

Prolonged hospitalization is another risk factor for increased CDI rates. Previous studies have indicated that the rate of CDI among certain hospitalized cancer patients is approximately twofold higher than that in general hospital patients because of longer hospital stays and extensive antibiotic consumption [119, 120]. Additionally, a higher rate of surgical procedures leads to longer hospital stays for recovery and subsequently contributes to an increase in the rate of CDI. Calu et al. observed that the incidence of CDI development in CRC patients with an age of >60 years and hospitalization was lengthened by about 53.8% [38]. In contrast, Fang et al. found that cancer patients ≥ 50 years old, with less than 10 days of hospitalization, had a *C. difficile*-positive rate of about 12.7%, while cancer patients ≤ 50 years old, with at least 10 days of hospitalization, exhibited a high rate of *C. difficile*-positive cases, reaching up to 35% [121]. These data suggest that patients with CRC experiencing longer hospitalization may be more susceptible to *C. difficile* acquisition, regardless of age. A systematic review demonstrated that, in general populations, older age was significantly associated with CDI incidence in hospitalized patients and was a likely risk factor for mortality. However, in specific populations, such as patients with cancer, age was often not related to CDI risk [111], indicating the higher importance of hospitalization than aging for CDI development. However, further evidence is required to clarify this aspect.

Bile acids metabolism

Bile salts play a pivotal role in the *C. difficile* life cycle. The bile salt cholate and the conjugated bile salts glycocholate and taurocholate trigger the germination of *C. difficile* spores, promoting CDI [122, 123]. Bile acid profiles may be affected by the components of the gut microbiota [19]. The primary bile acids, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), are typically conjugated to glycine or taurine and mostly (~ 95%) recycled in the liver, whereas the remaining enter the colon and are deconjugated and converted into secondary bile acids by the bacterial microbiota [124]. Among the gut microbiota components, the Firmicutes phylum, specifically the *Lachnospiraceae* and *Ruminococcaceae* families, has a positive correlation with secondary bile acids and plays an important role in bile acid metabolism, whereas members from the *Enterobacteriaceae* and *Lactobacillaceae* families have a negative correlation with secondary bile acids [125, 126]. Bacterial species with 7α-dehydroxylating activity, such as *Clostridium scindens*, can transform unconjugated primary bile acids into secondary bile acids, including deoxycholic acid (DCA) and lithocholic acid (LCA) [124]. In addition, *B. fragilis*, *Bacteroides vulgatus*, *Clostridium perfringens*, *Lactobacillus*, *Bifidobacterium* and *Listeria monocytognes* can also affect bile acid metabolism by producing BSH, which can conjugate both primary and secondary bile acids [127]. The use of broad-spectrum antibiotics decreases the microbiota diversity, resulting in reduced conversion of primary bile acids to secondary bile acids, with a subsequent increase in *C. difficile* growth and spore germination [122].

Bile acids may have a crucial impact on CRC initiation [128, 129]. High concentrations of bile acids damage the colonic epithelial cells and trigger the production of reactive oxygen species, leading to genetic instability and cancer stem cell-like formation [129]. Primary and secondary bile acids can interact with specific receptors, such as the Farnesoid X Receptor (FXR), which can activate signaling pathways related to inflammation and tumorigenesis. A pervious study found an increased level of secondary bile acid-producing bacteria in high-risk populations for CRC [130]. Cong et al. demonstrated that the gut microbiota could act as a negative regulator for CD8 + T cell effector function by altering the concentration of DCA. They reported that Bacteria harboring secondary bile acid biosynthetic genes, such as *C. scindens*, suppressed the CD8 + T cells effector function and promoted tumor growth in mice [131], demonstrating the key role of gut microbiota in bile acid metabolism and cancer development [132]. In addition, previous studies have reported that gut microbiota-mediated bile acid metabolism can affect the tumor microenvironment by affecting T helper cells

and regulatory T cells (Treg cells) [133, 134]. There is evidence of the involvement of bile acids in CRC progression by activating the epidermal growth factor receptor (EGFR) pathway, resulting in cell proliferation, p53 inhibition, invasion, and angiogenesis [135, 136]. In addition, some conditions, such as bile acid diarrhea (BAD), due to overproduction of bile acids or dysfunction or resection of the terminal ileum, increase colon concentrations of dihydroxy bile acids, triggering peristalsis and watery diarrhea, with an increased overall risk of cancer [137]. All these data suggest that bile acid metabolism dysregulation in patients with CRC may favor CDI development.

C. difficile colonization in patients with colorectal cancer

Several studies have investigated *C. difficile* colonization among patients with cancer [138, 139]. There are several data about *C. difficile* colonization in patients with CRC, with rates comprised between 33 and 35% (Table 1) [23, 83, 140]. These data suggest that weakening of the barrier of the gut microbiota due to cancer, together with a longer disease course and more aggressive treatment in patients with metastasis, might be a risk condition leading to *C. difficile* colonization. In addition, *C. difficile* colonization in CRC patients, especially during the adjuvant chemotherapy, seems to lead to more severe CDI able to compromise the ongoing chemotherapy itself [23]. Therefore, *C. difficile* colonization in pre-operative CRC patients might represent a risk for further cancer therapy, and further investigations are needed to clarify this aspect and the relationship between *C. difficile* colonization and CDI in cancer patients.

Incidence of *C. difficile* infection in patients with colorectal cancer

A history of cancer or malignancy per se is considered a risk factor for CDI. Therefore, it is not surprising that *C. difficile* is the most common pathogen associated with diarrhea in patients with cancer [27]. The incidence of CDI in recent years has been increasing despite the efforts to prevent this infection [79]. In a recent study, the incidence of CDI was estimated to be 1–2% in the hospitalized population and about 7–14% in adults with cancers [141], while the CDI incidence in cancer patients under chemotherapy is estimated to be approximately 7% [45]. Data from several studies indicated that CDI incidence rates in patients with CRC are comprised between 3.6% and 66.7% [26, 38, 60, 62, 140] (Table 1). Notably, Magat et al. found a higher level of anti-TcdB antibodies in the plasma samples from pre-operative CRC patients compared with healthy controls. These results demonstrate that the abundance of toxigenic *C. difficile* may be

Table 1 *Clostridioides difficile* rate of colonization and *C. difficile* infection (CDI) incidence in CRC patient

Study (n. of reference)	Sample size	Sample type	Study platform	Participants	*C. difficile* colonization rate (n. of strains)	% of CDI incidence (n. of strains)
Ong et al. 2024 [140]	522	Stool	RT-qPCR for GDH, TcdA, and TcdB	Post-operative CRC patients	6.7% (35)	5.17% (27)
Kim et al. 2021 [60]	1270	Stool	RT-qPCR and ELFA for TcdA and TcdB	Post-operative rectal cancer patients	–	3.6% (46)
Magat et al. 2020 [26]	39	Plasma	ELISA for anti-TcdB IgG	Pre-operative CRC patients	–	66.7% (26)
Calu et al. 2019 [38]	360	Retrospective study	ELISA for TcdA and TcdB	Post-operative CRC patients	–	7.77% (28)
Zheng et al. 2017 [23]	205	Stool	RT-qPCR and MLST	Pre-operative CRC patients	16.1% (33) - Positive lymph node (LN) metastasis patients (22.3%) LN negative (10.7%)	–
Zheng et al. 2016 [83]	206	Stool	RT-qPCR	Pre-operative CRC patients	16.0% (33) - Patients > 60 years old (18.2%) - Patients < 60 years old (12.2%)	–
Yeom et al. 2010 [62]	219	Stool	ELISA for toxins	CRC patient	–	6.8% (15) with *C. difficile*-associated colitis

CDI Clostridioides difficile infection, *CRC* colorectal cancer, *ELFA* enzyme-linked fluorescent assays, *ELISA* enzyme-linked immunosorbent assay, *GDH* glutamate dehydrogenase, *LN* metastasis, lymph node metastasis, *MLST* multilocus sequence typing, *RT-qPCR* quantitative reverse transcription polymerase chain reaction, *TcdA* toxin A, *TcdB* toxin B

associated with CRC progression [26]. In addition, higher CDI incidence rates have also been reported in postoperative CRC patients compared with controls [38, 60, 62]. However, few studies have investigated the incidence of *C. difficile* in patients with CRC [27], and more comprehensive epidemiological studies are needed to determine the importance of different risk factors involved in CDI development in CRC populations.

Colorectal cancer risk following *C. difficile* infection

There is evidence supporting that *C. difficile* is a plausible promoter of human CRC (Fig. 1C). Drewes et al. found that *C. difficile* strains derived from human colon cancer induced tumorigenesis in tumor-susceptible mouse models [50]. The results showed that tumor formation relied on *C. difficile* TcdB, which induced the activation of the Wnt–β-catenin pathway. This pathway plays critical roles in embryonic development, adult tissue homeostasis, and production of reactive oxygen species. In addition, β-catenin is a major regulator of cell proliferation and contributes to the formation of epithelial-mesenchymal transition (EMT), which is a well-known feature of cancer cell invasion, metastasis, and therapy resistance [142]. Previous studies have demonstrated that both TcdA and TcdB can activate NF-κB signaling pathway [33, 143]. NF-κB activity promotes tumor cell proliferation, suppresses apoptosis, and attracts angiogenesis [144]; therefore, these toxins may be involved in cancer development through activation of this signaling pathway.

Interestingly, TcdB can induce senescent cells; therefore, CDI could cause an accumulation of these cells that are characterized by long survival and that could push pre-neoplastic cells in the colon toward the complete neoplastic transformation in CRC by the senescence-associated secretory phenotype (SASP) [145]. Interestingly, a recent retrospective study reported that CRC incidence rate remained uniform over the entire study period (between 1990 through 2012) in patients negative for *C. difficile*, whereas it increased (about 2.7 fold) in patients positive for *C. difficile* within the first 4 years after *C. difficile* diagnosis, providing new evidence supporting that *C. difficile* is associated with an increased risk of CRC [146]. In contrast, another retrospective study (2010–2020) reported a decreased incidence of CRC in patients with a history of CDI compared with patients without a history of CDI, except for those obese cases for which the opposite was observed, suggesting that obesity, combined with CDI, might lead to increased inflammation in the intestine and increased risk of malignancy [147]. However, further research is warranted to explore the tumorigenesis role of toxigenic *C. difficile* strains.

Managing patients with colorectal cancer and *C. difficile* infection

CDI may be critical in CRC patients, especially during the administration of chemo drugs, resulting in the discontinuation of chemotherapy [67]. Prevention and rapid and accurate diagnosis of CDI are crucial for preventing infection and applying control strategies, particularly in patients at high risk for *C. difficile* acquisition.

Prevention

C. difficile transmission from person to person occurs via the oral-fecal route [20]. Symptomatic CDI patients are the main source of transmission, causing widespread contamination of the environment near the patients [148]. CDI prevention is a critical point for patients with cancer because this infection significantly increases the risk of mortality, prolonged hospitalization, and diarrhea, which is the most common symptom of CDI and leads to dose reductions in chemotherapeutic or radiotherapeutic regimens [28, 29]. There are several well-established guidelines to prevent CDI, most of which focus on preventing the transmission of *C. difficile* spores and reducing the susceptibility of patients to CDI [149]. Based on the CDC guidelines, practicing good hand hygiene is the best way to prevent the spread of *C. difficile* from one person to another [150]. The guidelines strongly recommend isolation of patients with CDI in private rooms [149]. In addition, regularly cleaning and disinfecting equipment and the environment of patients with CDI can kill the *C. difficile* spores and help reduce the risk of CDI [150]. Another approach is the implementation of a detection system that helps in the immediate diagnosis of *C. difficile* and is considered as an alert system to prevent CDI transmission [149]. The guidelines also propose several recommendations for reducing the susceptibility of patients to CDI, such as reduction of the unnecessary use of antibiotics and PPIs and administration of probiotics to increase in the diversity of the gut microbiota. Opioids and antimotility agents are recommended to be stopped in cancer patients with CDI because they may contribute to toxic megacolon [151, 152]. Furthermore, when CDI is associated with chemotherapy, switching to an alternative regimen usually leads to symptomatic improvement within 72 h [153]. Further studies are needed to determine a comprehensive protocol for the prevention of *C. difficile* in patients with CRC.

Diagnosis

Currently, several methods with different sensitivity and specificity are recommended for CDI diagnosis, including toxigenic culture (TC), cell cytotoxicity neutralization assay (CCNA), enzyme immunoassays (EIA) for TcdA and TcdB, glutamate dehydrogenase (GDH), and

nucleic acid amplification tests (NAATs) [43]. *C. difficile* culture techniques are highly sensitive but relatively slow. EIAs and NAATs are high-specificity tests for detecting TcdA/B but they have low sensitivity. GDH is a sensitive test for *C. difficile* detection but does not differentiate between toxigenic and non-toxigenic strains [154–156]. In general, and in particular, for oncologic patients, there are recommendations to use a diagnostic algorithm that combines more tests: a two-step algorithm, with the highly sensitive test used first, followed by confirmation using a highly specific test, or a three-step algorithm, that adds a NAAT test as a final step in unclear results [154, 157, 158].

Interestingly, Kamboj et al. found that the selection of diagnostic tests is extremely important for CDI diagnosis in cancer patients. In fact, the introduction of molecular-based testing methods for CDI in the Cleveland Clinic (Ohio) and Memorial Sloan Kettering Cancer Center (MSKCC) (New York) resulted in the duplication of the CDI rates in these patients [119]. In addition, immune checkpoint inhibitors (ICIs), a revolutionary cancer therapy that enhances antiantitumor activity by blocking negative regulators of T-cell function, are associated with immune-mediated diarrhea and colitis (IMDC) [159]. Therefore, due to overlapping symptoms, it may be difficult to interpret *C. difficile*-positive stool in the context of IMDC, leading to a potential underestimation of this infection. Accordingly, molecular-based testing methods in combination with a highly sensitive test can be recommended for CDI diagnosis in CRC patients. In addition, cytotoxicity assays can be used to detect the *C. difficile* toxins in fecal samples and monitor active disease.

Assessment of severity of *C. difficile* infection

According to the SHEA/IDSA guidelines, severe CDI is defined as a white blood cell (WBC) count > 15,000 cells/μL or a serum creatinine level ≥ 1.5 mg/dl [158]. These criteria have some limitations for cancer patients, who are usually neutropenic and have higher creatinine levels than the non-cancer population [74, 160]. For these reasons, several authors have proposed scales for severity markers in cancer patients. Zar et al. have proposed that CDI may be considered severe if cancer patients present with two or more of the following characteristics: age > 60 years, temperature ≥ 38.3 °C, an albumin level < 2.5 mg/dL, or a peripheral WBC ≥ 15,000 cells/μL within 48 h of hospitalization, endoscopic evidence of pseudomembranous colitis, or being in the intensive care unit (ICU) [161]. Differently, Belmares et al. have suggested that CDI may be considered severe if cancer patients show three or more of the following points: temperature ≥ 38.0°C, ileus (either clinically or radiographically), systolic BP < 100 mmH, leukocytosis with WBC ≥ 15,000/μL (one point)/WBC > 30,000/μL (two points), and abdominal CT scan one abnormal finding (one point)/≥ two abnormal findings (two points) [162]. Since these two detailed scales are expensive, including colonoscopy and CT scan, respectively, Yoon et al. have predicted CDI mortality in cancer patients considering severe neutropenia (absolute neutrophil count ≤ 500/μL) as associated with a worse outcome [163]. A prospective cohort study including 553 patients reported no statistically significant difference in 30-day all-cause mortality between cancer patients and those without cancer and concomitant CDI [164]. Notably, Yepez Guevara et al. demonstrated that cancer patients may experience more severe CDI episodes due to infection with virulent ribotypes, such as 027 [165]. Furthermore, cancer patients with CDI may experience an increased risk of treatment failure or recurrence compared with non-cancerous patients due to immunosuppression [45, 166]. Data on CDI recurrence (rCDI) rates among cancer patients remain scarce and are limited because of the small sample size and inadequate analysis of recurrent risk factors. A recent observational population-based cohort study comparing rCDI rates between patients with and without a cancer diagnosis found an increased risk of mortality in patients with cancer or a history of cancer as a secondary outcome [167]. Scappaticci et al. estimated the recurrence rate in hematology patients to be approximately 41% [141]. However, further studies considering large numbers of patients are needed to determine the rate of rCDI in patients with cancer.

There is discordant evidence of CDI severity in CRC patients. Some authors have reported more severe clinical symptoms of CDI in CRC patients than in healthy controls, as well as higher rates of complications, such as extended pre-operative hospital stay, admission to ICU, increased readmission rate, and mortality, compared with non-CDI patients [25, 168]. In contrast, Polpichai et al. found that CDI in CRC patients was associated with a longer length of hospitalization, increased incidence of peritonitis, bowel perforation, paralytic ileus, and colectomy, but was also associated with a lower risk of mortality, sepsis, septic shock, acute kidney injury, and mechanical ventilation, compared with patients without CRC [168]. These data may be partially due to the treating physicians' vigilance on early diagnosis and treatment of CDI in patients with CRC, which may contribute to the observed decreased mortality and end-organ damage in these patients, and surgical intervention that may contribute to better outcomes as well.

Treatment

Treatment plays a vital role in managing CDI in patients with CRC. Antibiotic therapy during the early stages of infection may prevent CDI progression and gut injury

development. Nevertheless, the use of broad-spectrum antibiotics can disrupt the gut microbiota, leading to a higher risk of recurrent CDI and potentially affecting cancer progression [50, 51]. Hence, the use of approaches that cause minimal damage to the microbiome composition and restore gut microbiota diversity may provide long-term efficacy for controlling infection or even help treat CRC. So far different approaches have been introduced for CDI, which exhibit high effectiveness and specificity with minimal side effects compared with standard therapies (Table 2). In recent years, most studies have focused on the application of specific antibiotics, antibody therapy, probiotics, and FMT [39, 42, 169–171], as detailed in the following sections.

Table 2 Efficacy and microbiota impact of standard and emerging therapies for *C. difficile* infection (CDI) and recurrent CDI (rCDI)

Treatment type	Clinical stage	Disease type	Intervention	Sustained cure rate	Recurrence rate	Impact on gut microbiota	References
Vancomycin (Standard therapy)	Approved in 1986	CDI	125 mg for 10 days (4 times a day)	64.1%	23–25%	- Decreased abundance of *Bacteroides*, *Prevotella*, *Clostridium coccoides*, and *Clostridium leptum* - Increased abundance of *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*	[172, 173]
Fidaxomycin (Standard therapy)	Approved in 2011	CDI	200 mg for 10 days (twice daily)	74.6%	11–15%	- Decreased abundance of *Anaerobutyricum* spp., *Anaerostipes* spp., *Coprococcus* spp., *Bifidobacterium* spp., and *Enterococcus* spp. - Increased abundance of *Bacteroides* spp., and *Escherichia* spp.	[172–175]
Bezlotoxumab (Antibody therapy)	Approved in 2016	rCDI	10 mg/Kg of body weight	64%	17%	–	[176]
REBYOTA (FMT)	Approved in 2022	rCDI	Single-dose containing 150 mL of microbiota suspension (about 10^7 CFU/mL)	70.6%	No recurrence after 6-month follow-up	- Decreased abundance of Gammaproteobacteria and Bacilli - Increased abundance of Bacteroidia and Clostridia	[58]
VOWST (FMT)	Approved in 2023	rCDI	Daily administration of capsule for 4 days	88%	12%	- Decreased abundance of proinflammatory *Enterobacteriaceae* - Increased abundance of Firmicutes (*Ruminococcaceae* and *Lachnospiraceae*)	[177]
Ridinilazole (Narrow-spectrum antibiotic)	Phase III	CDI	200 mg for 10 days (twice daily)	73.0%	8.1%	- No activity against gram positive bacteria - Low activity against *Eggerthella lenta*, *Bifidobacterium* spp, *Peptostreptococcus anaerobius*, and *Finegoldia magna*	[178, 179]

Specific antibiotics

Narrow-spectrum antimicrobial agents have the potential to control *C. difficile*, which helps conserve the commensal gut microbiota and subsequently reduce the risk of recurrence [180].

In 2018, the IDSA/SHEA guidelines no longer recommend metronidazole as a first-line treatment that can be used only when other first-line agents are not available [158]. A retrospective study on patients with hematologic malignancies and bone marrow transplants treated for CDI showed no significant difference among patients treated with metronidazole alone, vancomycin alone, or combination therapy [181].

The emergence of antibiotic-resistant bacterial strains has led to novel strategies that specifically target a bacterial species or strain, including clustered regularly interspaced short palindromic repeats (CRISPR), phage therapy, and narrow-spectrum antibiotics [40, 180, 182]. Regarding CDI treatment, in recent years, fidaxomicin, a narrow-spectrum antibiotic, has been introduced as a recommended first-line treatment for CDI, showing better outcomes in reducing recurrence risk than vancomycin [171]. Although patients with cancer responded more slowly to CDI treatment compared with non-cancer patients, resolution of diarrhea was more rapid with fidaxomicin than with vancomycin. In addition, fidaxomicin has shown higher cure rates and fewer recurrences in cancer population with CDI than vancomycin [166]. Moreover, fidaxomicin had less impact on gut microbiota composition and the loss of *C. difficile* colonization resistance compared with vancomycin [183].

Another novel narrow-spectrum agent is ridinilazole, which has been introduced for CDI treatment and is currently in a phase III trial [178]. Ridinilazole is highly effective in treating CDI in phase I and II clinical trials, superior preservation of the intestinal microbiota, and lower risk of CDI recurrence compared with vancomycin. The mechanism of action of ridinilazole is thought interfering with cell division. This antibiotic specifically targets clostridia without affecting other fecal bacteria [184, 185]. A previous study demonstrated that ridinilazole could help maintain bile acid metabolism and reduce the risk of recurrence associated with bile acid dysregulation [184]. There are no data on the application of ridinilazole for treating CDI in cancer population, and more evidence is needed to clarify the efficacy of this antibiotic in targeting CDI in cancer patients.

There are different preclinical studies on the effect of narrow-spectrum antimicrobial agents on CDI. A new type of tetracycline antibiotic called omadacycline has shown encouraging outcomes in combating *C. difficile* [186]. Recently, various bacteriocins have been identified as narrow-spectrum agents whose mode of action is closely related to the bacterial strains [187]. Thuricin CD, produced by *Bacillus thuringiensis* strains, targets *C. difficile* strains and shows comparable antimicrobial activity to fidaxomicin, vancomycin, and metronidazole, whereas it has no significant effect on gut microbiota composition in a human colon model [175, 188]. However, the efficacy of these agents should be examined in clinical studies.

Antibody therapy

Bezlotoxumab is a monoclonal antibody targeting the toxin B of *C. difficile*, that was approved by the FDA in 2016 and exhibits high efficacy in decreasing the incidence of rCDI in randomized clinical trials [189]. In a phase III clinical trial, bezlotoxumab showed a significantly lower rate of rCDI (around 40%) compared with placebo [176]. In addition, the use of Bezlotoxumab is recommended as an adjunct therapy to vancomycin for treating patients at high risk of recurrence or after a first recurrence [176, 190]. Recently, bezlotoxumab has shown promising results in reducing the rate of CDI recurrence in immunocompromised CDI patients and CDI patients with ulcerative colitis [191, 192]. There are no reports on the effectiveness of bezlotoxumab for treating CDI in patients with CRC, and only one study demonstrated that the use of bezlotoxumab reduced the rate of rCDI in cancer population [193]. However, this product has been removed from the US market due to commercial considerations. Intravenous immunoglobulin (IVIg) was previously used in clinical trial phases for treating patients with multiple rCDI, but it is now not recommended in the treatment guidelines [194, 195]. However, some clinicians continue to administer IVIg for severe rCDI cases [195]. Numerous in vitro and in vivo studies have been conducted on the use of anti-toxin antibodies to manage toxigenic *C. difficile* strains, which should be evaluated in clinical experiments [39].

Probiotics

Restoring gut microbiota and microbial-secreted metabolites is a promising strategy for combating CDI and CRC (Fig. 2). Probiotics are potential agents for treating various diseases by restoring the gut microbiota or influencing the host immune system. Probiotics can help prevent and treat different diseases through various mechanisms, such as enhancement of gut barrier functions, inhibition of colonization of pathogenic bacteria, and immunomodulation [196]. Furthermore, current clinical studies on probiotic therapy have demonstrated the effectiveness of probiotics in improving surgical or chemotherapy outcomes in cancer patients, most of which have focused on the impact of lactobacilli and bifidobacteria [197, 198]. A randomized study (NCT03782428) found that the oral administration of a probiotic cocktail, including

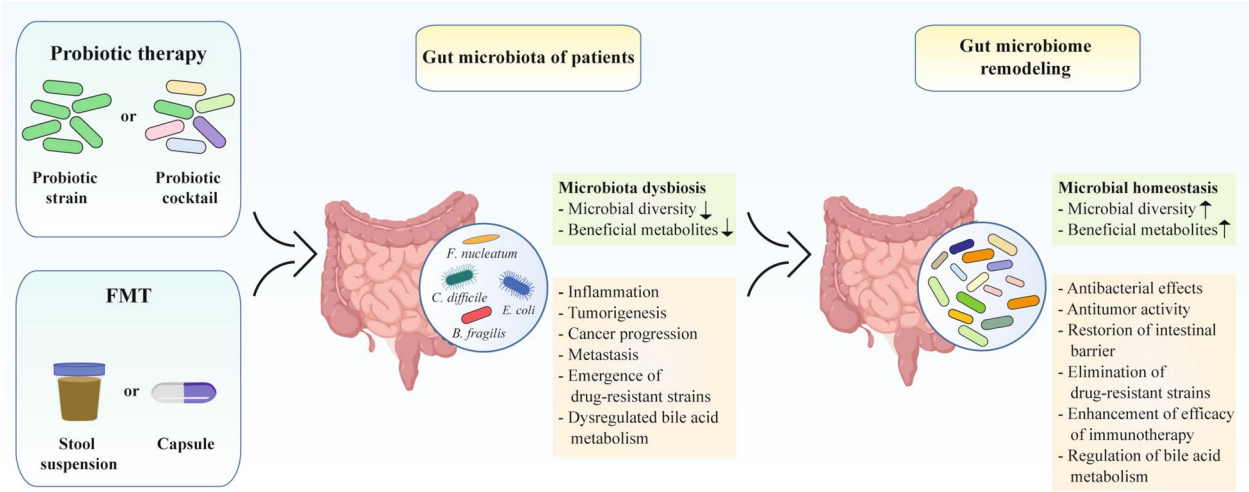

Fig. 2 Schematic representation of gut microbiota modulation using probiotic therapy or FMT. The gut microbiota of patients with CRC or CDI have low bacterial diversity, which leads to a decrease in beneficial bacterial metabolites such as SCFAs and an increase in drug-resistant bacterial strains. This state can elevate the inflammation, tumorigenesis, and overgrowth of opportunistic bacteria like *C. difficile*. Restoring gut microbiota using probiotic administration or FMT helps increase in diversity of bacterial species, resulting in improving the balance of microbiota and mucosal barriers and restoring gut homeostasis. In addition, modulation of gut microbiota can increase the antitumor activity and efficacy of immunotherapy. *CDI Clostridioides difficile* infection, *CRC* colorectal cancer, *SCFAs* short-chain fatty acid, *FMT* fecal microbiota transplant

strains of lactobacilli and bifidobacteria, decreased the production of proinflammatory cytokines such as TNF-α, IL-17A, IL-17C, IL-22, and IL-12, and prevented post-surgical complications in CRC patients [199]. The use of probiotic formulations, such as OMNi-BiOTiC R© 10 AAD, as adjuvant therapy to reduce diarrhea associated with FOLFIRI-based chemotherapy in patients with metastatic CRC is under investigation in a phase II trial (NCT03705442). Additionally, the efficacy of the combination of VE800 (an 11-strain probiotics) with nivolumab in patients with metastatic CRC is currently in a phase I/II trial (NCT04208958) [200]. Some of these clinical trials have focused on the role of metabolites produced by microbiota in the treatment of CRC. For example, the results of a clinical trial (NCT03072641) indicated that the use of a mixture of *B. lactis* and *Lactobacillus acidophilus* increased the abundance of butyrate-producing bacteria in the mucosal and fecal samples of patients with CRC [201].

The most common probiotics used for treating CDI have been investigated as a supplementary treatment. A randomized controlled trial of probiotic capsules containing *L. acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *B. lactis* Bi-07, and *B. lactis* Bl-04 improved diarrhea outcomes and the incidence of diarrhea in adult CDI patients compared with placebo. This probiotic therapy did not affect the rate of rCDI compared with placebo [202]. Furthermore, a randomized clinical trial showed that combining antibiotic therapy with probiotic therapy prevented diarrhea and CDI in ICU patients [203]. A systematic review with meta-regression analysis demonstrated that probiotics can decrease the risk of CDI in hospitalized patients by more than 50% when used after the initial antibiotic dose [204].

The use of probiotics in patients with cancer is a controversial topic. Osterlund et al. reported that colon cancer patients receiving active 5-Fu chemotherapy treated with daily *Lactobacillus* supplementation showed a decrease in the diarrhea grade, abdominal pain, and hospital stay [205]. Similarly, Benchimol et al. reported that treatment with metronidazole and probiotics resolved CDI in a patient with leukemia [206]. Differently, Cohen et al. reported that 0.5% of hematopoietic stem cell transplantation (HSCT) patients treated with *lactobacillus* developed bloodstream infection mainly due to this bacterium [207]. Therefore, there are questions concerning the safety, timing, and dose of probiotics for use in patients with cancer that need to be investigated.

Fecal microbiota transplantation (FMT)

FMT has been introduced as a direct method for restoring the composition of gut microbiota. FMT is a procedure that delivers minimally manipulated fecal microbiota from a healthy donor to a recipient with a specific disorder [41]. FMT is an FDA-approved procedure for the treatment of rCDI with an effectiveness of approximately 90% and is recommended to treat rCDI patients non-responding to fidaxomicin or vancomycin [208]. This method successfully restores the diversity of the gut microbiota and the metabolic landscape and

regulates bile acid metabolism [209]. However, there are concerns about the long-term safety of FMT in terms of the risk of the transfer of antibiotic-resistant genes and pathogens from donor to recipient, and this needs to be investigated further [210]. For example, colibactin-producing *pks*+*E. coli* promotes colon tumorigenesis in a mouse model, and colibactin-specific mutational signatures identified in human organoids match those observed in 5–10% of human CRC tumors [211]. Nooij et al. investigated changes in the prevalence and abundance of potentially carcinogenic *pks*+*E. coli* after FMT and found that the pks status of patients treated with FMT depended on the pks status of the donor (P = 0.046) [212]. Although current screening protocols for FMT donors are safe, routine screening for *pks* is not required. However, because it is unknown how long pks^+ *E. coli* may persist in cured rCDI patients and over what time frame this may contribute to CRC, further studies are needed to evaluate the cancer risk due to pks^+ *E. coli* in patients with rCDI and other patients.

Two live biotherapeutics, namely Rebyota™ (fecal microbiota live-jslm; RBX2660; RBL) and VOWST (fecal microbiota spores, live-brpk; SER-109], were approved in 2022–2023 by the US Food and Drug Administration (FDA) for the treatment of rCDI [41]. Rebyota is a frozen mixture of microbes from human feces that is delivered to the recipient via enema. The phase III trial of Rebyota demonstrated the efficacy of this drug in reducing the rate of rCDI after antibiotic treatment compared with placebo (70.6% vs. 57.5% success rate, respectively) [58]. Vowst is a consortium of viable purified Firmicutes spores that are administered orally over 3 consecutive days following bowel preparation and taken on an empty stomach. The results of the phase III trial of Vowst demonstrated that the administration of Vowst after standard antibiotic therapy was superior in reducing recurrence compared with placebo (88% and 60% success rate, respectively) [213].

Several clinical studies are exploring the use of FMT in treating patients with CRC, especially those who have received CRC immunotherapy, to enhance the efficacy of therapy (Table 3). Zhao et al. demonstrated that combining FMT with tislelizumab and fruquintinib enhances survival in refractory microsatellite-stable (MSS) metastatic CRC, supporting the effectiveness of FMT in treating this group of patients [214].

Data available indicate that FMT as a treatment for CDI in immunocompromised patients, including patients with cancer, have comparable efficacy and safety to those for patients with a healthy immunity system, although further randomized trials including these patient populations would be necessary [215]. In particular, FMT in patients with cancer appears to be a safe and effective treatment for CDI, with no instances of bacteremia or Cytomegalovirus (CMV) seroconversion due to FMT [216]. These data are of considerable importance considering that higher rCDI rates have been reported in cancer patients compared to non-cancer patients [141, 164]. The effectiveness of FMT on rCDI in patients with hematologic cancer receiving chemotherapeutic agents was observed by Hefazi et al., who reported an effective rate of 86% without serious side effects or infectious

Table 3 FMT protocols for CRC patient under clinical trials

NCT number	Sponsor	Sample size	Immunotherapy agent (s)	Stage	Administration/recipients
NCT04729322	M.D. Anderson Cancer Center	15	Nivolumab and Pembrolizumab	Phase II	- Pretreatment with metronidazole, vancomycin, and neomycin - Colonoscopic FMT followed by capsule administration up to 6 months - MSI-H/dMMR CRC patient; who failed at least 2-dose anti-PD-1/PD-L1
NCT05279677	Chinese Academy of Medical Sciences	30	Sintilimab and Fruquintinib	Phase II	- Microbiota capsules for 8 cycles - Chemo refractory mCRC patients
NCT04208958	Vedanta Biosciences, Inc	56	Nivolumab	Phase II/I	- Pretreatment with vancomycin - Daily VE800 (11 commensal bacterial strains) every 4 weeks - Advanced or mCRC patients
NCT04130763	Peking University	10	–	Phase I	- Microbiota capsules for 3 days - mCRC patients, who failed at least 2-dose anti-PD-1/PD-L1
NCT06205862	Shenzhen Hospital of Southern Medical University	466 (estimated)		Phase II	- Colonoscopic FMT - Recurrence of CRC patients

CRC colorectal cancer, *FMT* fecal microbiota transplant, *mCRC*, metastatic colorectal cancer; MSI-H/dMMR, microsatellite instability-high/mismatch repair deficient; NCT number, National Clinical Trial number;−1, Programmed cell death protein 1; PD-L1, programmed death-ligand 1

complications [170]. Additionally, Ali et al. reported FMT as an effective treatment for rCDI in cancer patients, even in those receiving cancer treatment or immunosuppressive therapy, although the authors observed a long-term benefit from FMT in only 74% of cases, probably due to multiple coexisting risk factors such as malignancy itself, cancer therapies used, immunocompromised condition, and frequent antibiotic use [217].

Discussion

Cancer patients face a higher risk of developing CDI because of factors like aging, undergoing surgery, chemotherapy, antibiotic therapy, and hospitalization. These risk factors are associated with an alteration of gut microbiota composition, generally characterized by higher levels of Proteobacteria and Fusobacteriota and lower levels of abundance of Bacteroidetes, such as *Bacteroides,* and Firmicutes, such as *Ruminococcaceae* [218], which provides a favorable environment for the germination of *C. difficile* spores and CDI development [219]. An imbalance in the gut microbiota also results in an alteration of the metabolites produced by intestinal bacteria, which can also affect *C. difficile* colonization and infection [126, 136, 220]. For example, the production of SCFAs, such as butyrate, propionate, and acetate, by *Bacteroides* and *Ruminococcaceae* can stimulate the secretion of secretory IgA and inhibit *C. difficile* adherence and growth. These metabolites also exert protective effects against CRC progression [221]. Furthermore, an imbalance in the gut microbiota results in the alteration of bile acid metabolism and enrichment of primary bile acids, promoting the growth of *C. difficile* cells [126, 136]. Interestingly, the alteration in abundance *Lachnospiraceae* and *Ruminococcaceae* families that confirmed the low abundance in CRC, had a positive correlation with the concentration of secondary bile acids and resistance to *C. difficile* [18]. Therefore, the depletion of these bacteria plays a critical role in the development of CDI in CRC patients [222].

The severity and recurrence rate of CDI can be higher in the cancer population compared to other patients, thereby affecting overall survival [25]. Nonetheless, research on the role of *C. difficile* in cancer development, the prevalence of *C. difficile*, and the rCDI rate in cancer patients remains relatively scarce, and current studies are limited to research with a small sample size; therefore, more comprehensive research is required to elucidate the exact relationship between *C. difficile* and CRC. In addition, careful management of CDI in oncology patients is critical to minimize complications.

In patients with CRC, an appropriate diagnostic algorithm that combines two or three different assays with high specificity and sensitivity for *C. difficile* is strongly recommended [154, 223] to determine whether a positive result represents a colonization or an active infection by *C. difficile*, since pre-operative CRC patients and those with advanced disease are frequently *C. difficile* colonized [23]. Current guidelines for diagnosing and treating CDI are based on disease severity [224]. However, due to the peculiar characteristics of cancer patients, different scales of CDI severity markers have been proposed [161–163]. There is discordant evidence on the severity of CDI in patients with CRC [25, 137] that requires further investigation and analysis of data from a larger number of patients.

A low impact of CDI treatment on the gut microbiota of CRC patients is necessary because microbiota dysbiosis may play a role in the promotion or progression of CRC, as well as in the increase of rCDI rates [56, 57]. In general, a selection of appropriate antibiotics and a proper dosage may not only be effective for treating CDI but also for preventing CDI development [37]. Currently, the use of fidaxomicin has been recommended for treating CDI in patients with CRC, and further research is needed to explore the utility of more selective antibiotics targeting *C. difficile*, which specifically modulate the abundance of this bacterium in patients with cancer, such as ridinilazole. It should be noted that the emergence of antibiotic-resistant strains highlights the urgent need for global antibiotic stewardship and infection control efforts [225]. Hence, the detection of new antibiotic resistance patterns of bacteria may help clinicians choose treatment approaches and develop novel strategies for controlling pathogens.

Among the available treatments, gut microbiota modulation by probiotics or FMT can be an effective approach for treating CRC patients with CDI and improving CRC outcomes. The use of probiotics has shown favorable results for treating CRC and CDI [199, 202, 203]. However, further research is needed to determine the optimal strain and dosage for ensuring the prevention and treatment of CDI in patients with CRC and their ability to prevent and treat CDI in these patients. Moreover, it is important to consider the potential of probiotic strains to restore gut microbiota composition when selecting for therapeutic purposes. The effects of several bacterial species, such as *B. fragilis*, on gut microbiota restoration have previously been demonstrated [226]. Further studies are needed to explore the potential of probiotics to specifically promote gut microbiota in the future.

FMT is a highly effective technique for restoring the gut microbiota and treating rCDI [191, 208]. FMT appears to be a safe and effective treatment for CDI in patients with cancer, without serious side effects or infectious complications [215–217], representing an effective approach for treating CDI, rCDI, and improving outcomes in patients with CRC or metastatic CRC [191, 208]. In addition,

FMT can help regulate bile acid metabolism and restore SCFA levels in patients post-FMT [227]. Notably, SCFAs can play critical roles in bile acid metabolism and exert protective effects against CDI by regulating bile acid metabolism [228].

FMT has been successfully used as a supplement to immunotherapy, helping improve outcomes in patients with CRC or metastatic CRC [214]. The use of immunotherapeutic agents such as pembrolizumab and nivolumab (programmed cell death 1 (PD1)-blocking antibodies), has shown high efficacy in metastatic CRC patients with mismatch-repair-deficient and microsatellite instability-high (dMMR–MSI-H) [229], and data about the application of FMT for CRC treatment are limited to this group [196]. However, randomized clinical studies with larger sample sizes and diverse patient populations are needed to further explore the efficacy of FMT, the consequences of its usage, and its effectiveness in treating CRC patients with CDI.

In conclusion, CDI may be a complication in CRC patients due to sharing similar risk factors, affecting the duration of hospitalization, increasing the recurrence rate, altering the response to therapy, and increasing mortality. Therefore, early diagnosis and treatment of CDI are essential for reducing the burden of CDI-related complications in these patients, especially in population at high risk of *C. difficile* acquisition, such as those undergoing prolonged hospitalization. Further epidemiological studies on the precise prevalence and clinical correlates of *C. difficile* in the CRC population are needed to manage these complications. Additionally, comprehensive protocols are needed to establish effective preventive interventions and monitor the incidence of *C. difficile* in patients undergoing cancer therapy. A diagnostic algorithm that combines two or three assays with high specificity and sensitivity should also be considered to detect *C. difficile* in cancer patients. Among the available treatments, microbiota manipulation may represent a hopeful strategy for the recovery of gut microbiota and management of CDI in patients with CRC, although future studies are needed to provide more evidence about the mechanisms of action of this approach and its effectiveness in treating CDI in patients with CRC.

Abbreviations

APCs	Antigen-presenting cells
BAD	Bile acid diarrhea
BFT	*Bacteroides fragilis* toxin
BSH	Bile salt hydrolases
CA	Cholic acid
CCNA	Cell cytotoxicity neutralization assay
CDADC	*Difficile*-associated diarrhea
CDC	Centers for disease control and prevention
CDCA	Chenodeoxycholic acid
CDI	*Clostridioides difficile* infection
CDT	*C. difficile* transferase
CRC	Colorectal cancer
CRISPER	Clustered regularly interspaced short palindromic repeats
DCs	Dendritic cells
DCA	Deoxycholic acid
EGFR	Epidermal growth factor receptor
EIA	Enzyme immunoassays
ELFA	Enzyme-linked fluorescent assays
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
FMT	Fecal microbiota transplant
FXR	Farnesoid X receptor
GDH	Glutamate dehydrogenase
GI tract	Gastrointestinal tract
HSCT	Hematopoietic stem cell transplantation
ICIs	Immune checkpoint inhibitors
ICU	Intensive care unit
IDSA	Infectious diseases society of America
IL	Interleukin
IMDC	Immune-mediated diarrhea and colitis
IVIg	Intravenous immunoglobulin
LCA	Lithocholic acid
LN metastasis	Lymph node metastasis
mCRC	Metastatic colorectal cancer
MLST	Multilocus sequence typing
MSI-H/dMMR	Microsatellite instability-high/mismatch repair deficient
MSKCC	Memorial sloan kettering cancer center
MSS	Microsatellite-stable
NAATs	Nucleic acid amplification tests
NCT number	National clinical trial number
NFATc	Nuclear factor of activated T cell C3
NF-κB	Nuclear factor κB
NHDS	National hospital discharge survey
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PMC	Pseudomembranous colitis
PPIs	Proton pump inhibitors
rCDI	Recurrent CDI
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SCFAs	Short-chain fatty acids
SHEA	Society for healthcare epidemiology of america
TC	Toxigenic culture
TcdA	Toxin A
TcdB	Toxin B
TNF-α	Tumor necrosis factor alpha
WBC	White blood cell
Wnt	Wingless-related integration site

Author contributions

H.R. reviewed the literature and drafted the manuscript, S.P., A.S., and F.B. reviewed and critically edited the manuscript; G.T., H.S, M.R.Z., and E.N.M. revised the manuscript. All authors read the final version of the manuscript and approved the list of authors.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This declaration is not applicable.

Competing interests

The authors declare no competing interests.

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