MIRJournal
Microbiology Independent Research Journal

REWIEW

DOI: 10.18527/2500-2236-2021-8-1-10-17

Clostridium difficile infection in the pediatric patients of an oncological hospital: cultivation of anaerobic intestinal flora and treatment

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ABSTRACT

In recent years, the number of infectious diseases caused by *Clostridium difficile* in the world has grown with a significant increase in relapses and mortality in patients, particularly among cancer patients in hospitals. An increase in the resistance of *Clostridium difficile* to first-line drugs, namely metronidazole and vancomycin, has also been observed and that makes the search for new methods of treatment and the prevention of this infection even more urgent. In this review, we analyze the recent data on the methods of the cultivation and isolation of the pure bacterial culture of *Clostridium difficile* and other anaerobic enteropathogens over the course of enterocolitis treatment with antimicrobial drugs in pediatric patients with oncopathology. Novel approaches to the therapy of this infection are discussed.

Keywords: pediatric oncology, *Clostridium difficile* infection, anaerobic bacteria

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Citation: Shvydkaya MG, Dzhandarova DT, Mitrokhin SD. Clostridium difficile infection in the pediatric patients of an oncological hospital: cultivation of anaerobic intestinal flora and treatment. MIR J 2021; 8(1), 10–17. doi: 10.18527/2500-2236-2021-8-1-10-17.

Received: December 24, 2020

Accepted: January 18, 2021

Published: April 5, 2021

Conflict of interest: The authors declare no commercial or financial conflicts of interests.

The urgency of the problem

In recent years, an increase in the number of infectious diseases as well as relapses and mortality caused by *Clostridium difficile* (*Clostridium difficile* infection, CDI) has been observed among the cancer patients admitted to hospitals worldwide [1]. The increasing urgency of Clostridia detection in Russia is associated with the identification of severe *Clostridium difficile* (*C. difficile*) diarrhea cases with hemi-colitis [2]. It is especially important for immunosuppressed patients since they are a risk group for developing severe forms of CDI [3]. The incidence of CDI is growing among children [4] and has already reached 25% [5]. The detection rate of *C. difficile* toxins in children was 37.4% in a multidisciplinary hospital in Russia in 2016 [6]. *C. difficile* strains play a particularly important role in the development of diarrhea in cancer patients [7]. It is known that CDI develops primarily in people who belong to high-risk groups: people with immunodeficiency as well as patients in hospitals or in closed long-term care facilities [8]. Patients of a pediatric oncological hospital have a combination of several risk factors for the CDI development: leukemia as the underlying disease, treatment with immunosuppressants and/or cytostatics, and antibiotic treatment [9].

A two-stage approach is recommended for the diagnosis of CDI infection [10]. The gold standard diagnostic for CDI infection is the cultivation of toxigenic bacterial strains. The problems with the cultivation of anaerobic flora are associated with an increase in the resistance of

C. difficile strains to antibacterial drugs [11]. In order to test the sensitivity of *C. difficile* to antibacterial drugs, it is necessary to isolate a pure bacterial culture first. Improvement of the cultivation methods will simplify the seeding of toxigenic *C. difficile* strains and make it a routine and accessible method in every bacteriological laboratory.

The etiology of a large percentage of the diagnosed diarrhea cases remains unknown demonstrating the need for improved laboratory diagnostics. [12]. This is especially true for children undergoing therapy in an oncological hospital since the treatment of a concomitant disease disrupts the intestinal mucosa and can lead to sepsis [13]. The situation is complicated by the lack of protocol for detecting opportunistic anaerobic intestinal flora in oncological hospital settings in Russia. The bacteriological method covers a wide range of opportunistic flora, which is etiologically significant in the structure of diarrhea in children – patients of an oncological hospital.

The clinical guidelines of the Russian Federation include two registered drugs for the treatment of CDI – metronidazole and vancomycin [10]. Metronidazole is recommended for the treatment of an uncomplicated disease, while vancomycin is the drug of choice for the treatment of severe and recurrent CDI. At the same time, the administration of vancomycin leads to a higher incidence of CDI relapses than treatment with fidaxomicin, which is currently not registered in the Russian Federation [14]. The data analysis showed an increase in *C. difficile* resistance to the first-line drugs in CDI therapy [11], which makes the search for new methods of treatment and the prevention of this infection urgent.

Cultivation of toxigenic *C. difficile* strains

The choice of diagnostic tests to confirm CDI is controversial due to the variety of laboratory methods used in different institutions and the lack of a single standard protocol. The differences in sensitivity, specificity, duration, and cost of different diagnostic methods have led to the implementation of different algorithms for the CDI diagnosis confirmation in different labs. The optimal approach to the laboratory diagnosis of CDI remains an unsolved problem [15].

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommended the use of a two-stage algorithm for the diagnosis of antibiotic-associated diarrhea [15]. On the other hand, M. A. Sukhina et al. recommend the use of a three-step algorithm based on the use of immunological, bacteriological, and molecular biological methods. This ensures a timely diagnosis, local microbiological monitoring, and the epidemiological surveillance of *C. difficile*-associated infection [16]. At the same time, the cultivation of toxigenic strains for diagnostic purposes is recommended in controversial cases when the other tests show conflicting results. Therefore, there is no single approach in the diagnosis of CDI, and each laboratory must evaluate and determine the necessary multi-step algorithm, suitable for a specific patient population [15]. Unfortunately, this is difficult in routine practice in Russia due to the lack of funding and equipment that often leads to the implementation of only one method, which significantly reduces efficiency.

The culture method is characterized by high sensitivity and specificity and, in combination with other methods, enables the determination of resistance to antibacterial drugs. Its application is hampered since it requires the use of special equipment, but it is impossible to abandon this method completely [10]. In order to prescribe adequate and effective therapy for Clostridial infection, it is necessary to reliably identify the type of isolated pathogen and obtain an antibiotic chart, since different *Clostridium* species vary significantly in their sensitivity to antimicrobial drugs [17]. All this emphasizes the demand to modify and simplify the cultivation technique.

The pretreatment of native material contributes to spore preservation and the inactivation of the accompanying flora. Therefore, when using the heat shock method on native feces, only spores remain in the material, and the growth of the accompanying microflora is excluded [18]. However, the best results were achieved by the subsequent introduction of the samples treated by the heat shock into the storage broth. At the same time, the highest percentage of pure culture by direct seeding on selective agar without the preliminary addition to the storage broth was obtained using the method including the preliminary treatment of the material by alcohol shock [19]. The use of selective media with cefoxitin and cycloserine inhibits the growth of other *Clostridium* species [18]. The enrichment of the medium with brain heart broth and the addition of 0.5% yeast extract, 0.1% l-cysteine, cycloserine-cefoxitin, and 0.1% sodium taurocholate, followed by cultivation on agar containing 7% defibrinated horse serum and 0.1% taurocholate, provides a more sensitive and selective combination for detecting low concentrations of *C. difficile* in samples [20]. Chromogenic cycloserine-cefoxitin media are considered as the most sensitive and convenient. However, at the moment, there is no cultivation method that gives a 100% sowing rate.

Several algorithms were developed in order to reduce the multi-stage cultivation process. The use of ChromID *C. difficile* agar (CDIF, bioMérieux, France) and PRO disc (PRO disc K1532B, Key Scientific Products,

USA) for untreated stool samples in combination with the Gram stain procedure makes it possible to isolate and identify *C. difficile* strains in 98.3% of cases [21]. Storage media can be combined with other methods since the high bacterial concentration increases the sensitivity of the glutamate dehydrogenase test [16]. The use of a storage broth prior to running the indirect reaction of the neutralization of cytotoxin leads to an increase in the sensitivity of detecting of toxigenic strains of *C. difficile* [22].

C. difficile is also found in the stool of children with diarrhea caused by other pathogens [23]. The etiological factor of diarrhea in a pediatric oncological hospital could be represented not only by the toxigenic strains of *C. difficile* but also by other anaerobic microorganisms that can cause an infectious process at various locations [24]. It is known that anaerobic microorganisms can cause severe hospital infections. This is especially true for immunosuppressed children, since flora from the gastrointestinal tract can enter the bloodstream through intestinal barriers that have been disturbed in the course of treatment of the underlying disease. This can cause microbial translocation from the intestine and lead to systemic disease [25].

It is noteworthy that the problem of dysbiosis in malignant neoplasms has not been sufficiently studied – the studied populations are heterogeneous and the research methodology is ambiguous. Moreover, only a few studies have been devoted to microbiological and clinical changes in microflora in children with cancer [25].

The spectrum of the anaerobic intestinal flora of patients in a pediatric oncological hospital in Russia remains poorly studied. In recent years, the literature describes a number of diarrhea cases with etiological factors caused by anaerobic microorganisms, such as *Bacteroides* sp. [26], *Clostridium perfringens* [27], and *Clostridium butyricum* [28]. Camorlinga et al. [29] showed that nontoxigenic *C. difficile* strains can be cytotoxic. At the same time, it is necessary to use the specific growth media or test systems for the diagnosis of each nosology. The problem of modern CDI diagnostics is that, at present, cultivation methods do not allow the routine sowing of any anaerobic opportunistic intestinal flora by means of one universal technique. Before the start of therapy for the underlying disease in the oncological hospital every patient undergoes screening tests for a number of infections, including CDI while the other anaerobic bacteria are ignored. The presence of anaerobic opportunistic flora in the intestine of one patient in a children's hospital can lead to the infection of other immunosuppressed patients, which in turn can cause serious consequences.

New treatments for the CDI

The common approach for the therapy of CDI in pediatric oncology is the use of antibiotics such as metronidazole and vancomycin [30]. These antibiotics are registered in Russia for the treatment of CDI and are recommended by the National Association of Infections Control specialists that is associated with the health care organizations [10]. However, the emergence of *C. difficile* strains resistant to these antibiotics requires new approaches to treat this nosology. For example, the narrow-spectrum drug fidaxomicin – a macrolide class antibiotic – has been approved by the U.S. Food and Drug Administration for the treatment of CDI patients including children [31]. However, this drug is not currently registered in the Russian Federation.

In addition to the standard methods of treatment, there are several alternative and unconventional antimicrobials for CDI that are in different testing stages at present. These include the following:

- teicoplanin, glycopeptide class antibiotic, reduces the infection recurrence rate compared to vancomycin [32];
- tigecycline, a potential antibiotic for the treatment of CDI, especially in severe cases [33];
- ridinilazole, an antibacterial drug that is as effective as vancomycin; it shows potential in the treatment of initial stages of CDI and provides a sustained effect by reducing disease recurrence [34];
- ramoplanin, an antimicrobial lipoglycopeptide in phase 2 trials for the treatment of CDI;
- ribaxamase (SYN-004), a β-lactamase; it can prevent *C. difficile* infection in patients receiving intravenous β-lactam antibiotics without disrupting the gut microbiome [35].

However, the clinical trials data on the effectiveness of the above-listed drugs are contradictory, and these drugs can currently be considered as an addition to the main traditional CDI therapy [36, 37, 38].

There are drugs that have successfully passed phase 2 clinical trials but did not show significant results in phase 3. For example, surotomycin – a lipopeptide antibiotic – has shown good efficacy, but did not show any advantage over vancomycin in the treatment of CDI [39]. Cadazolid, an oxazolidinone-fluoroquinolone, showed good activity against *C. difficile*, but did not show better efficacy in comparison with vancomycin according to the results of the last study [40]. Perhaps these results could be better explained by the imperfect study design than by the low effectiveness of the drugs themselves. However, this study confirms that the positive results obtained from intermediate steps do not always coincide with a positive end result.

The antibiotic-free treatment approach involves understanding how *C. difficile* strains and their toxins

interact with the human microbiota and immune system. Spore suppression is one of the interesting directions for the development of the new therapeutic strategy for CDI. Howerton et al. demonstrated that the bile salt analog CamSA inhibits the germination of *C. difficile* spores in mice [41]. However, it remains to be seen whether CamSA can be used as an effective anti-spore agent in human therapy. The use of another analogue of bile acids – ethaverine, that is approved in European Union – is limited in therapeutic practice, since its mechanism of action lies in binding to the *C. difficile* toxin TcdB, rather than TcdA [42] and does not affect sporulation. Direct use of bile salts, such as taurocholate, tolevamer, cholestyramine, and colestipol, is recommended for the treatment of CDI, although the mechanisms of their interaction with *C. difficile* strains as well as their effect on the microbiome are not well understood. On the other hand, a recent study has demonstrated the problems with using bile salts for therapy [43]. The results of a study by Ueda et al. [44] showed that tetramic acid derivatives produced by *Pseudomonas aeruginosa* exhibit high activity against *C. difficile*, although these results have not been confirmed in clinical settings yet and require further research. The reasons for the failure may lie in an insufficient study of the microbiome, its changes, and the interaction of microorganisms with the development of a particular infection.

One of the leading therapies for CDI cases without complications is the use of probiotics, such as *Saccharomyces boulardii* [7], but medications containing live microorganisms are contraindicated for immunosuppressed patients. As a result, feces transplantation (intestinal administration of donor flora obtained from feces) as well as the use of bacteriophages [45] are limited in a pediatric oncological hospital. For the same reason, the application of nontoxigenic strains of *C. difficile* is not a suitable treatment for the prevention or cure of CDI. In addition, this approach is associated with the risk of horizontal gene transfer from the bacterial pathogenicity loci and the transformation of nontoxigenic *C. difficile* bacteria into toxigenic strains [46].

The use of intravenous immunoglobulins (IVIG), monoclonal antibodies, and vaccination in order to reduce the risk of developing CDI was suggested in a number of studies. However, the data collected at present are insufficient for the recommendation of these methods for the treatment of CDI in children – patients of an oncological hospital [47].

Promising drugs that need further studies as treatment options for CDI include:

- auranofin, effective against *C. difficile* M7404 *in vitro*; may become an ideal therapeutic option for the treatment of CDI in the future [48];
- GyrB inhibitor DS-2969b, active against *C. difficile in vitro* and *in vivo*, does not disrupt the microbiome, and is characterized by a low incidence of resistance [49];
- antibiotics of the acyldepsipeptide class, their mechanism of action at present is poorly understood [50];
- rhodomyrtone – a bioactive compound obtained from the leaves of pink myrtle (*Rhodomyrtus tomentosa*) – causes lysis of *C. difficile* vegetative cells and prevents the proliferation of spores more effectively than vancomycin [51];
- ebselen, has bactericidal activity against *C. difficile*, inhibits the production of toxins and sporulation [52].

These new drugs have a number of advantages over classical CDI treatments, but they are currently at an early stage of development and no definitive conclusions can be drawn until clinical trials are completed.

Therefore, the problem of laboratory diagnostics and the treatment of CDI in pediatric oncology is of pressing interest. The microbiological method for CDI diagnostics is still relevant in pediatric oncology, although currently it is not the leading method. Medications that have fast action, low relapse rate, preserve the intestinal microbiome, and lack resistance to the active substance of *C. difficile* strains are in the first priority group of drug candidates in the search for new methods of the treatment and prevention of CDI. Children in the oncology hospital represent a population that requires a special approach for the treatment of CDI. To date, an effective algorithm for the treatment of CD infection in children – patients of a pediatric oncological hospital – has not been developed.

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