

REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

***Clostridium difficile*-associated diarrhea in infants and children**Biljana Vuletić^{1,2}, Elizabeta Ristanović³, Slavica Marković^{1,2}, Zorica Rašković^{1,2}, Vladimir Radlović⁴, Zoran Igrutinović^{1,2}¹Clinical Centre Kragujevac, Pediatric Clinic, Serbia;²University of Kragujevac, Faculty of Medical Sciences, Serbia;³Military Medical Academy, Belgrade, Serbia;⁴University Children's Hospital, Belgrade, Serbia**SUMMARY**

Clostridium difficile (CD) is the most common cause of nosocomial diarrhea in adults with high rates of morbidity and mortality. The epidemiology of CD infection (CDI) has changed in the last few decades associated with increasing severity of the infection rate related to the occurrence of NAP1 hypervirulent strain and the emergence of the disease among ambulatory patients and the wider community. Although little is known about CDI in pediatric patients, CD is surprisingly recognized as an important pathogen in children. In this review article, we direct attention to the recent findings on the incidence and epidemiology of pediatric CDI, including the risk factors for infection, with special emphasis on the importance of CDI in infants and a population of children suffering from chronic gastrointestinal diseases or cancer. Despite recent pharmacotherapeutic protocols successfully used in children with CDI, we would like to draw attention to precautionary and preventive measures in terms of both unnecessary testing and uncritical use of antibiotics as the most important risk factors.

Keywords: *Clostridium difficile*; diarrhea; children; epidemiology; treatment

INTRODUCTION

Clostridium difficile (CD) was first reported as a cause of diarrhea in 1978 [1]. It is an anaerobic Gram-positive bacterium, widely distributed in nature, and its spores can be found in soil, water, and human and animal feces. It normally exists in the intestinal flora in 3% of healthy adults and in 30–50% of infants, and the carriage rate in children and adults is about 20% [2]. CD exists in the non-toxic form and does not cause disease. Pathogenic strains produce different types of toxins and the best known are enterotoxin A and cytotoxin B, both of which cause diarrhea and inflammation known as *Clostridium difficile* colitis in infected patients. In addition, life threatening systemic effects such as ascites, pleural effusion, cardiopulmonary arrest, hepatic abscess, abdominal compartment syndrome, multiple organ dysfunction syndrome and renal failure have been reported as part of the disease [3].

EPIDEMIOLOGY

It has long been believed that *Clostridium difficile* infection (CDI) is a disease of adults and is not typical for the pediatric population, despite a high-rate gastrointestinal colonization after birth. Findings obtained during the period from 1997 to the present day indicate that

CDI is increasingly found as a cause of diarrhea in children, both inside and outside hospital. Independently of the fact that infant testing is not advised, 26% of hospitalized children with CDI were less than one year old and as many as 5% were newborns. Large epidemiological studies covering a period of 2001–2006 in 22 children's hospitals in the United States show the increase of admissions with CDI from 2.6 to 4.0/1,000. The mean age of these patients was four years, and about 25% were less than one year old [4]. The increasing trend continued, which led to the unexpected fact that CD is a very important pathogen in children with a still unclear explanation and the dilemma of whether this is the result of changes in the ecology of microorganisms or better diagnostics [5, 6, 7]. To find the answer to this question, the emergence of a hypervirulent strain NAP1/ribotype 027 (North American pulsed – field gel electrophoresis type 1) is very significant. This strain isolated in several states of the USA and Europe possesses altered *tcdC* sequences in the inhibitor gene that produces the protein suppressing A/B toxin production. Although NAP1 strain is the one most responsible for severe forms of CDI, 2 NAP7 and NAP8 have also been isolated with insufficiently tested mechanism of action [8, 9]. Risk factors for CDI are well known: hospitalization and administration of antibiotics, but since the disease occurs in outpatient conditions as well and without

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previous antimicrobial therapy, it has become clear that other parameters also contribute to the development of infection. These are the use of proton pump inhibitors, prolonged placement of a nasogastric tube, previous abdominal surgery, renal failure, immunosuppression, and previously known gastrointestinal diseases, particularly cancer and post-transplantation conditions [10].

***Clostridium difficile* in newborns and infants**

The neonatal intestinal tract is sterile, but as early as in 12-month-old newborns it is more like in adults. CD carriers are, on average, 37% of infants and 30% of 1/6-month-old newborns. This colonization is slightly affected by the vaginal membrane rupture or previous use of antibiotics, and the environmental conditions of infants are also particularly important. CDI can be transmitted by the hands of hospital personnel, bathtubs, thermometers, and changing tables. Breast-feeding babies are considerably less colonized with CD (14%) if compared to formula-fed babies (30%). At the age of 6–12 months, about 14% of children are CD carriers, and the percentage among three-year-olds is similar to non-hospitalized adults and amounts to 0–3%. Clinically, the disease rarely occurs before the age of 12–24 months, and one of the reasons is a deficient cellular mechanism of toxin binding at this age [9, 11, 12]. In the intensive care unit, 7% of patients with diarrhea and 15% of controls were colonized with CD [13]. In two studies of children 0–2 years of age, 11–59% of those with diarrhea and 24–33% of controls were colonized with CD [14, 15]. Among children 0–34 months of age, 21% of those with diarrhea and 33% of controls were carriers of CD [16]. Due to the high rate of asymptomatic carriage, CD toxin detection cannot be the major cause of diarrhea before adolescence, especially in young children and infants [17, 18].

CD infection is transmitted via fecal-oral route. Highly resistant spores cannot be destroyed by alcoholic disinfectants; they survive for long in the environment and are entered by hand, contaminated food and water, in both hospital settings and outside. Once ingested, acid-fast spores pass the stomach and after exposure to bile acids they germinate into vegetative forms in the colon, producing toxins A and B, which cause diarrhea and pseudomembranous colitis. The process is favored by the imbalance in the intestinal flora resulted mostly from long-term antibiotic treatment. The effect of the toxins leads to necrosis in surface epithelium and to the creation of a pseudomembrane consisting of fibrin and inflammatory infiltrates composed mainly of polymorphonuclear cells. Hemorrhages are detected in the damaged submucosa but without inflammatory infiltrates as in ulcerative colitis and bacillary dysentery. In the most severe cases of pseudomembranous colitis, pseudomembranes may be detached, necrosis may extend to deeper layers of the colon wall and penetrate through serosa into the peritoneal cavity.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

The clinical picture of CDI has different manifestations ranging from asymptomatic colonization or mild diarrhea to fulminant disease accompanied by a clinical picture of toxic megacolon and sepsis with a potentially lethal outcome. A mild form of the disease involves the emergence of two to three watery stools with abdominal pain and discomfort in the abdomen. A severe form of CDI in children appears with over 15 watery stools per day, an increase in body temperature, severe abdominal pain, loss of appetite, blood or pus in the stool followed by the loss of body weight [19]. CDI diagnosis is based on the clinical symptoms of diarrhea, and a positive finding of stool toxins. Enzyme immunoassay has become the most commonly used technique for detection of enzymes A and B with sensitivity of 72–82%, and specificity of 97–98%. Less available tests approved by the FDA – nucleic acid amplification tests (NAATs), including the PCR technique – are rapid, sensitive, and of similar specificity if compared to enzyme immunoassay. Since asymptomatic carriers in childhood are so frequent, routine stool CDI testing in children less than one year old should be avoided. It may be considered in children one to three years of age with clinical signs of diarrhea and previously tested for other pathogens, particularly viral ones [20, 21]. In children over three years of age, the testing is carried out in cases of prolonged diarrhea when all other causes have been considered and risk factors are present. Endoscopic findings of pseudomembranes and hyperemic rectal mucous membranes are sufficient for the diagnosis of CDI at any age. It is advisable to test children with suspected megacolon, infants with chronic diarrhea, children with already detected diseases of the intestinal tract (Hirschsprung's disease, cancer), and those with immune deficiency [22]. It is particularly important to say that none of the CDI stool tests are recommended as a therapy audit. Excretion of toxins in 13–24% lasts for more than two weeks, and in 6% of cases up to four weeks after treatment [23–26].

TREATMENT

Discontinuation of antimicrobial therapy is the first step in treating CDI. Metronidazole is the drug of choice in children with mild and medium-to-severe forms of the disease. It is administered at a dose of 30 mg/kg with a maximum dose of 2 g per day. Oral vancomycin or vancomycin via rectal enema along with/without intravenous metronidazole is administered in the initial treatment of patients with a severe form of infection and to those who did not respond to oral metronidazole. Severe or fatal disease is possible in neutropenic children with leukemia, intestinal tract disease (Hirschsprung's disease) and chronic inflammatory bowel diseases. The treatment should last up to 10 days. From experience, the resistance of CD to metronidazole is extremely low, even when it comes to the newly isolated strain NAP 1 [10, 27–30]. Patients with recurrent disease (about 30%) usually respond well to repeated treatment. In case of the second relapse of infections, metronidazole is

not used as a chronic treatment for its potential neurotoxicity. Under the circumstances, the drug of choice is vancomycin. Other antimicrobial drugs used in the treatment of CDI are nitazoxanide, fidaxomicin (approved for adults by the FDA in 2011) and rifaximin but without sufficient evidence of application in children [28–30]. In treating CDI, the so-called biotherapy deserves a prominent role, including the use of probiotics (*Saccharomyces boulardii*, *Lactobacillus species*), as well as fecal transplants and immunoglobulins [31–33]. It has been shown that infusing healthy donor feces has been significantly more effective in the treatment of recurrent CDI if compared to vancomycin (Netherlands Organization for Health Research and Development and the Netherlands Organisation for Scientific Research) [34]. The data about the application of intravenous immunoglobulins and monoclonal antibodies against A/B CD toxins are based mainly on studies in adult patients [35]. Preliminary attempts of parenteral CD vaccine containing A/B toxoids show high-rate antibody formation in serum of healthy adult subjects. In a reported case, three patients were on continuous vancomycin therapy for persistent recurrent CDI. They were all able to discontinue the therapy and in two of them a significant increase in serum antibodies against toxin A and toxin B was recorded [35]. Surgery for CDI involves subtotal or total colectomy and is recommended only in patients who had not responded to medical therapy and in those with a clinical picture of fulminant colitis. Such complication is relatively rare in children and its manifestations are poor general condition, signs of systemic infection, intense abdominal pain, and colonic distension with CT colonography. Under these circumstances, diarrhea may not be present due to ileus.

REFERENCES

1. Khanna S, Pardi DS. Clostridium difficile infection: new insights into management. *Mayo Clin Proc.* 2012; 87(11):1106–17.
2. McFarland LV, Brandmarker SA, Guandalini S. Pediatric Clostridium difficile: a phantom menace or clinical reality? *J Pediatr Gastroenterol Nutr.* 2000; 31(3):220–31.
3. Steele J, Chen K, Sun X, Zhang Y, Wang H, Tzipori S, et al. Systemic dissemination of Clostridium difficile toxins A and B is associated with severe, fatal disease in animal models. *J Infect Dis.* 2012; 205(3):384–91.
4. Kim J, Smathers SA, Prasad P, Leckerman KH, Coffin S, Zaoutis T. Epidemiological features of Clostridium difficile-associated disease among inpatients at children's hospitals in the United States, 2001–2006. *Pediatrics.* 2008; 122(6):1266–70.
5. Nylund CM, Goudie A, Garca JM, Fairbrother G, Cohen MB. Clostridium difficile infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med.* 2011; 165(5):451–7.
6. Zilberbeng MD, Tillotson SG, McDonald LC. Clostridium difficile infections among hospitalized children, United States, 1997–2006. *Emerg Infect Dis.* 2010; 16(4):604–9.
7. Cohen SH, Gerding DH, Johnson S, Kelly CP, Loo VG, McDonald LC, et al. Clinical practice guidelines for Clostridium difficile infection in adults; 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect Control Hosp Epidemiol.* 2010; 31(5):431–45.
8. Warny M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, et al. Toxin production by an emerging strain of Clostridium difficile associated with outbreaks of severe disease in North America and Europe. *Lancet.* 2005; 366(9491):1079–84.
9. Schutze GE, Willoughby RE; Committee on Infectious Diseases; American Academy of Pediatrics. Clostridium difficile infection in infants and children. *Pediatrics.* 2013; 131(1):196–200.
10. Sammons JS, Toltzis P, Zaoutis TE. Clostridium difficile infection in children. *JAMA Pediatr.* 2013; 167(6):567–73.
11. Enoch DA, Butler JM, Pai S, Aliyu SH, Karas JA. Clostridium difficile in children: colonisation and disease. *Journal of Infect.* 2011; 63(2):105–13.
12. Jangi S, Lamont T. Asymptomatic colonization by Clostridium difficile in infants: implication for disease in later life. *J Pediatr Gastroenterol Nutr.* 2010; 51(1):2–7.
13. Boening DA, Fleisher GR, Campos JM, Hulkower CW, Quinlan RW. Clostridium difficile in a pediatric outpatient population. *Pediatr Infect Dis.* 1982; 1(5):336–8.
14. Elis ME, Mandal BK, Dunbar EM, Bundell KR. Clostridium difficile and its cytotoxin in infants admitted to hospital with infectious gastroenteritis. *Br Med J.* 1984; 288(6416):524–6.
15. Mårdh PA, Helin I, Colleen I, Oberg M, Holst E. Clostridium difficile toxin in faecal specimens of healthy children and children with diarrhoea. *Acta Paediatr Scand.* 1982; 71(2):275–8.
16. Vesikari T, Isolauri E, Maki M, Grönroos P. Clostridium difficile in young children. Association with antibiotic usage. *Acta Paediatr Scand.* 1984; 73(1):86–91.
17. Radlović N, Leković Z, Vuletić B, Radlović V, Simić D. Acute diarrhea in children. *Srp Arh Celok Lek.* 2015; 143(11–12):755–62.
18. Vuletić B, Obradović S, Stojković-Andelković A, Igrutinović Z, Radlović P. Rotavirusni gastroenteritis. *Srp Arh Celok Lek.* 2006; 133(3–4):166–9.

PREVENTION

Since CDI is becoming more and more significant in pediatrics, there is a need for increased supervision in the maintenance of hygiene in hospitals and stricter environmental controls [36]. Contact precaution includes regular hand washing with soap and water, particularly during diarrhea, which is much more efficient than alcohol-based products to which the bacillus is resistant. Wipes soaked with 10% sodium hypochlorite may be useful, while gloves provide the best protection in controlling transmission. It should be borne in mind that the skin of CDI patients is contaminated in the groin, chest, abdomen, forearm, and hand, and that spores can be easily transmitted to medical personnel. Skin contamination persists on the abdomen and chest even after the resolution of diarrhea [37]. A recent extensive meta-analysis of 26 randomized controlled trials found that supplementation with probiotics is associated with significant reduction in the risk of CDI at all ages (children and adults), both hospitalized and non-hospitalized [32].

CONCLUSION

Dramatic changes in the epidemiology of CDI have clearly shown the growing trend of diseases among children lately and persuade both pediatricians and institutions to perform preventive action, the former of which in terms of rational use of antimicrobial therapy in order to reduce the most important risk factor and avoid unnecessary testing, and the latter of which in terms of increased supervision in compliance with hygiene measures.

19. Sunenshine RH, McDonald LC. Clostridium difficile-associated disease: new challenges from an established pathogen. *Cleve Clin J Med.* 2006; 73(2):187–97.
20. Vuletić B, Obradović S, Ristanović E, Kostić G, Radlović P. Molekularna epidemiologija rotavirusa u dece predškolskog uzrasta. *Srp Arh Celok Lek.* 2005; 133(11-12):482–3.
21. Vuletić B, Radlović N, Leković Z, Ristić D, Mladenović M, Pavlović M, et al. Ishrana deca sa rotavirusnim gastroenteritisom. *Hrana i ishrana.* 2008; 49(1-4):30–6.
22. Igrutinovic Z, Peco Antic A, Radlovic N, Vuletic B, Markovic S, Vujic A, et al. Pseudo Barrer Syndrome in an infant with Congenital Chloride Diarrhoea. *Srp Arh Celok Lek.* 2011; 139(9-10):677–80.
23. Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): Data review and recommendations for diagnosing Clostridium difficile-infection (CDI). *Clin Microbiol Infect.* 2009; 15(12):1053–66.
24. Toltzis P, Nerandzic MM, Saade E, O'Riordan MA, Smathers S, Zaoutis T, et al. High proportion of false-positive Clostridium difficile enzyme immunoassays for toxin A and B in pediatric patients. *Infect Control Hosp Epidemiol.* 2012; 33(2):175–9.
25. Wilcox MH, Planche T, Fang FC, Gilligan P. What is the current role of algorithmic approaches for diagnosis of Clostridium difficile infection? *J Clin Microb.* 2010; 48(12):4347–53.
26. Luna RA, Boyanton JR, Mehta S, Courtney EM, Webb CR, Revell PA, et al. Rapid stool-based diagnosis of Clostridium difficile infection by real-time PCR in a children's hospital. *J Clin Microbiol.* 2011; 49(3):851–7.
27. Locher HH, Caspers P, Bruyère T, Schroeder S, Pfaff P, Knezevic A, et al. Investigations of the mode of action and resistance development of Cadozolid – a new antibiotic for treatment of Clostridium difficile infections. *Antimicrob Agents Chemother.* 2014; 58(2):901–8.
28. Mattila E, Arkkila P, Mattila PS, Tarkka E, Tissari P, Anttila VJ. Rifaximin in the treatment of recurrent Clostridium difficile infection. *Aliment Pharmacol Ther.* 2013; 37(1):122–8.
29. Eyre DW, Babakhani F, Griffiths D, Seddon J, Del Ojo Elias C, Gorbach SL, et al. Whole-genome sequencing demonstrates that fidaxomicin is superior to vancomycin for preventing reinfection and relapse of infection with Clostridium difficile. *J Infect Dis.* 2014; 209(9):1446–51.
30. Joksimović Z, Bastić D. Clostridium difficile infekcija: prikaz novijih saznanja. *Timočki medicinski glasnik.* 2013; 38(1):50–60.
31. Goldenberg JZ, Ma SS, Saxton JD, Martzen MR, Vandvik PO, Thorlund K, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *Cochrane Database Syst Rev.* 2013; 5:CD006095.
32. Lau CS, Chamberlain RS. Probiotics are effective at preventing Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. *Int J Gen Med.* 2016; 9:27–37.
33. Gens KD, Elshabouty RH, Holt JS. Fecal microbiota transplantation and emerging treatments for Clostridium difficile infection. *J Pharm Pract.* 2013; 26(5):498–505.
34. Van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Eng J Med.* 2013; 368(5):407–15.
35. Lowy I, Molrine DC, Leay BA, Blair BM, Baxter R, Gerding DN, et al. Treatment with monoclonal antibodies against Clostridium difficile toxins. *N Engl J Med.* 2010; 362(3):197–205.
36. Pant C, Deshpande A, Altai MA, Minocha A, Sferra TJ. Clostridium difficile infection in children: a comprehensive review. *Curr Med Res Opin.* 2013; 29(8):967–84.
37. Bobulsky GS, Al-Nassin WN, Rigga MM, Sethi AK, Donskey CJ. Clostridium difficile skin contamination in patients with C. difficile-associated disease. *Clin Infect Dis.* 2008; 46(3):447–50.

***Clostridium difficile* дијареја код одојчади и деце**

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САЖЕТАК

Clostridium difficile (CD) најчешћи је узрок болничке дијареје одраслих са високом стопом морбидитета и mortalитета. У последњих неколико декада епидемиологија CD инфекције (CDI) промењена је са порастом стопе озбиљности инфекције у релацији са појавом хипервирулентног соја NAP1, као и појавом болести међу амбулантним пацијентима и у широј животној заједници. Иако је мало познато о CDI код педијатријских болесника, CD је изненађујуће препознат као важан патоген у децем узрасту. У овом прегледном чланку осврћемо се на скорашња сазнања о инциденци и

епидемиологији CDI код деце укључујући ризико факторе за инфекцију са посебним освртом на значај CDI код одојчета и у посебној популацији деце са хроничним болестима гастроинтестиналног тракта или канцером. Упркос новијим фармакотерапијским протоколима којима се са успехом третирају CDI код деце, скрећемо пажњу на све мере превенције и предострожност како у погледу непотребног тестирања тако и на некритичну примену антибиотика као најважнијег фактора ризика.

Кључне речи: *Clostridium difficile*; дијареја; деца; епидемиологија; лечење