Recent changes in *Clostridium difficile* infection
Recentes mudanças da infecção por *Clostridium difficile*

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ABSTRACT

Clostridium difficile is the main cause of nosocomial diarrhea. Diarrhea associated with *C. difficile* has increased incidence, morbidity, and mortality in the last few years. The major related risk factors include use of antibiotics, elderly patients and prolonged hospital stay. Many patients receive combinations of antibiotics or multiple antibiotics, which represents the main risk to develop diarrhea associated to *C. difficile* or its recurrence. Therefore, interventions to improve antibiotic prescribing, as well as compliance with infection control measures can reduce hospital-acquired *C. difficile* infections. This review addresses the epidemiological changes in *C. difficile* disease and its treatment.

Keywords: *Clostridium difficile*; Diarrhea/drug therapy; Anti-bacterial agents/therapeutic use; Risk factors

RESUMO

Clostridium difficile é a principal causa de diarreia hospitalar. A diarreia por *C. difficile* aumentou sua incidência e sua morbiletalidade nos últimos anos. Os principais fatores de risco relacionados são uso de antibióticos, idosos e permanência hospitalar prolongada. Muitos pacientes recebem combinação de antibióticos ou múltiplos antibióticos, constituindo-se, assim, o principal fator de risco para o desenvolvimento de infecção ou de recorrência de diarreia associada ao *C. difficile*. Por isso, intervenções que otimizem a prescrição de antibióticos associado à aderência de medidas de controle de infecção podem reduzir aquisição dessa infecção. Assim, esta revisão aborda a mudança da epidemiologia da infecção por *C. difficile* e seu tratamento.

Descritores: *Clostridium difficile*; Diarreia/quimioterapia; Antibacterianos/uso terapêutico; Fatores de risco

INTRODUCTION

Clostridium difficile (CD) was first described in 1935[1]. It is a Gram-positive sporulated bacteria, difficult to be grown in the usual culture media, therefore called *difficile*[2].

It is the main cause of diarrhea at hospitals. The cost attributed to it in the United States varies from US$ 2,470.00 to US$ 3,669.00 per episode[3,4]. The number of cases of CD increased in the last few years due to the indiscriminate use of antibiotics, higher number of immunosuppressed patients, of elderly people and a high rate of hospital occupation, favoring spore dissemination at hospitals[5].

The main risk factor associated to CD is the previous use of antibiotics. It was initially attributed to the use of clindamycin[2]. All antibiotics are currently related to the development of CD[2]. Severe outbreaks have been registered since 2000 in the United States (US), Canada and United Kingdom, with a high mortality rate that ranges from 6.9 to 16.7%[6].

Epidemiological changes were due to appearance of a new strain in the beginning of 2000[5,7,8]. During the first semester of 2004, Loo *et al.* analyzed 12 hospitals in Quebec, Canada, and detected an incidence of 22.5 cases per 100,000 hospital admissions[7]. Similar cases in other Canadian and American hospitals presented pseudomembranous colitis and fulminant colitis, with a higher recurrence rate[9,10].

This new strain is more virulent, producing a binary toxin characterized by the gene NAP1/BI/027, which produces 16-fold more toxin A and 23-fold more toxin B when compared to the more common strains[3]. This strain is also resistant to gatifloxacin as well as moxifloxacin[3].

Another change is represented by CD outbreaks in the community in patients with no known risk factors, such as pregnant or puerperal women and children, which develop severe conditions with high morbidity and mortality[11].

Risk factors

The main risk factors associated to CD are age older than 65, use of laxatives, proton pump inhibitors or H2 histamine as gastric protection, chemotherapy, renal failure, gastrointestinal surgery, nasogastric tube, mechanical ventilation, prolonged hospital stay and previous antibiotic therapy[10,12].

Many such factors are found in patients in Intensive Care Units (ICU), with a proper environment for higher incidence and hospital dissemination [13].

Historically, clindamycin, cephalosporines and penicillins are the antibiotics related to *Clostridium difficile*-associated diarrhea (CDAD)[14]. Recently, fluoroquinolones have been implicated to this infection[15].

Particularly, the use of proton pump inhibitors and histamine H2 increase gastric ph, favoring bacterial colonization and proliferation in the intestinal lumen[16]. Therefore, the use of those drugs associated to antibiotics potentiate the risk developing of CD[6].

Pathophysiology

CD transmission is fecal-oral, from person to person, from fomites, and from hospital furniture[6]. Spores remain in the environment for long periods and are resistant to the use of commercial disinfectants, favoring propagation at hospitals[14].

The spores survive in gastric acidity, germinate in the colon and there initiate toxin production[16]. Colonization and binding of CD to the intestinal mucosa is facilitated by the use of antibiotics changing the balance of intestinal microbiota[12,16]. The production of toxin A and B stimulates the production of tumor necrosis factor and interleukins, and increases vascular permeability[6]. Toxin A is responsible for activating and recruiting inflammatory mediators, and toxin B has direct cytotoxic effect[6].

The intense inflammatory process results in the destruction of intestinal *lamina propria,* blocking nutrients absorption, generating malabsorption and bacterial translocation [6].

Asymptomatic patients

The only natural reservoir for CD is human beings[16]. Many patients are colonized by CD and do not have symptoms[16]. Asymptomatic patients may present positive toxin for CD in stool tests[17]. The incidence in the general population is around 4%, and in inpatients, up to 20%[16]. The longer the hospital stay, the higher the risk of colonization by CD[16,18].

Those people are the reservoir for the transmission to those who are susceptible, and they remain asymptomatic because there is a balance between the normal microbiota and CD[19]. The trigger for growth of CD is the release of toxins and the use of antibiotics[19].

In those people colonized by CD, the specific humoral response is fast, and the level of IgG antibodies specific for the toxins is higher when compared to non-colonized patients[19].

There is no benefit in treating those people, since after a few weeks of treatment for CD, most patients will recolonize[20].

Clinical diagnosis

Clinical symptoms usually appear a few days after beginning antibiotics and may appear up to two months after discontinuation[6]. The exact incubation period is not known[6]. McFee et al. demonstrated that the mean time for onset of CD infection after hospital discharge is 20.3 days[6].

The definition of diarrhea in hospital environment is very important. Diarrhea is usually defined as passing stools twice a day or more[17]. The symptoms of CD infection are broad, including from mild symptoms, with a few diarrheic episodes and no fever, to severe conditions, with important toxemia[17].

Typical symptoms are aqueous diarrhea many times a day, improving after elimination, low fever, and leukocytosis[17]. Complications include hypoalbuminemia, dehydration and malnourishment[17]. The most severe cases coincide with a decrease in diarrhea due to atony and thinning of the intestinal mucosa, characterized by toxic megacolon that may progress to perforation[17].

Laboratory diagnosis

Stool culture is the gold-standard for diagnosis, with sensitivity close to 100% but it is not used due to its cost, to being labor intensive, and to the fact that the results take long to be obtained (mean of 48 hours)[21]. False positive results occurs in 10% of cases, including asymptomatic carriers[9].

The detection of toxin in stool culture is less sensitive than traditional stool culture and its sensitivity is 70%[9].

The most used laboratory methods are immune enzyme assays, with results in up to 2 hours. Nevertheless, depending on the exam methodology, sensitivity may vary between 50 and 99%, and specificity from 70 to 100%[9].

Another highly sensitive and specific method is polymerase chain reaction, sensitivity being higher than

90% and specificity of 100%. The comparison between the laboratory methods is displayed in table 1[9].

Colonoscopy is indicated in colitis when no toxin is found in the feces and a quick diagnosis is necessary[15]. It examines the whole colon, but may have colonic perforation as a complication.

Abdominal X-ray is unspecific; abdominal computed tomography (CT) helps to determine the extent of the lesion in more advanced cases, as well as to assess pneumoperitoneum and colonic perforation[15].

Cure criteria

Cure criteria is based on signs and symptoms cessation[13,14]. No laboratory method available should be used to assess follow-up, since they may remain positive for weeks or months, not necessarily implying disease recurrence[13].

Treatment

If possible, systemic antibiotics should be discontinued in patients with CD infection[22]. Mullane *et al*. analyzed patients using systemic antibiotics that acquired CD infection and concluded that in cases in whom antibiotics were discontinued, compared to the groups where they were maintained, the cure rate was higher, and relapse lower[22]. Gould et al. also demonstrated that relapse was 41% in patients receiving systemic antibiotics during the treatment for CD infection[20].

The main antibiotics used to treat of CD infection are metronidazole and vancomycin[21]. The advantage of the former one is low cost, good availability, and few side effects[23]. Nevertheless, failure with relapse occurs in 28% of all cases, specially due to the NAP1/BI/027 strain[23]. The main factors associated to relapse are patients with *diabetes mellitus*, sepsis, and previous surgery[23].

Vancomycin is no longer considered the treatment of choice for CD, since it has the same efficacy as metronidazole. There is the risk, during treatment, of having vancomycin-resistant *Enterococcus* spp[18]. It is indicated in patients with severe infection or who relapse[18]. The main treatment proposed for CD infection is described in chart 1.

Chart 1. Antimicrobial treatment for *Clostridium difficile* infection, based on severity[6]

Severity classification	Treatment
Mild or moderate diarrhea, leukocytes < 15.000/μl	Metronidazole 500 mg, PO, TID, for 10 to 14 days
Severe (fever, intense diarrhea, abdominal pain, leukocytes > 150.00/μl, increased creatinine)	Vancomycin 125 to 250 mg, PO, QID, for 10 to 14 days
(Hypotension, shock, toxic megacolon, paralytic ileus)	Vancomycin 500 mg, through nasogastric tube and/or enema, QID, with or without metronidazol, 500 mg IV every 8 hours

Zar et al. verified in their study that the patients who had mild CD infection presented the same cure rate when comparing metronidazole or vancomycin[24]. In patients with complicated CD, the cure rate with metronidazole was 76% and with vancomycin, 97%[24]. Also the recurrence rate was higher in the patients who used metronidazole[24].

Surgery with total colectomy is indicated in rare cases, in severe colitis with significant toxemic symptoms. Adjuvant treatment is enema with vancomycin 500 mg dissolved in 100 mL of saline and/or intravenous metronidazol[17,25]. Mortality may be as high as 60%[25].

Other treatment options
Fidaxomicin

Fidaxomicin (OPT-80) is an antibiotic more active *in vitro* than vancomycin, even for treating NAP1/BI/027 strains[22]. The recommended dose is of 200 mg/day every 12 hours, for 10 days[22]. It has minimal intestinal absorption, high fecal concentration, and does not change intestinal microbiota[22]. Response rate is similar to vancomycin with lower recurrence in strains other than NAP1/BI/027 (16.9 *versus* 29.2%)[22].

Rifaximin

Derived from rifamycin, it is active against Gram-negative, Gram-positive, and anaerobic agents[18]. It is not absorbed by the intestine, has high colonic

Table 1. Comparison of the different laboratory methods for diagnosis of *Clostridium difficile* infection in feces[9]

Assay	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Immune-enzyme	86.7 (68.4-95.6)	98.5 (95.3-99.6)	89.7 (71.5-97.3)	98 (94.6-99.4)
PCR	100 (85.9-100)	96.5 (92.6-98.4)	81.1 (64.3-91.4)	100 (97.6-100)
Cytotoxicity	90 (72.3-97.4)	97 (93.2-96.8)	81.8 (63.9-92.4)	98.5 (95.2-99.6)
Stool culture	100 (85.9-100)	92.9 (88.2-95.9)	68.2 (52.3-80.9)	100 (97.5-100)

CI: confidence interval; PCR: polymerase chain reaction.

concentration, is highly active against CD, and has a lower relapse rate [18]. The recommended dose is 400 mg, TID, for 10 days[18].

Nitazoxanide

Nitazoxanide is an antiparasitic drug which *in vitro* inhibits CD strains[26]. Freeman et al. verified *in vitro* that CD strains less sensitive to metronidazole were sensitive to nitazoxanide[27]. Some studies demonstrated that nitazoxanide was as efficient as metronidazole in the treatment of patients with CD[17,26]. The recommended dose is 500 mg, BID, for 10 days[17].

Immunotherapy

There are case reports according to which the use of intravenous unspecific immunoglobulin will benefit patients in recurrence, but there is little data in the literature[14].

Probiotics

Probiotics are found in fermented milk, yogurt, powders and capsules as lactobacillus, bifidobacteria and *Saccharomyces boulardii*[19]. They act inhibiting bacterial adhesion to the intestinal mucosa[19]. The efficacy of this agent in prevention is controversial, since the studies are heterogeneous[19]. It is still inconclusive whether they are effective in prevention or could be used as adjuvant treatment in primary infection or in relapse episodes[19].

RECURRENCE

Recurrence occurs more often during the first or second week after the end of the treatment[6]. Around 25% of patients may relapse, even after being treated with vancomycin[6]. If the patient has more than two relapses, the risk for a new episode increases to 50 to 65%[6].

Recurrence occurs due to the spores that remain in the intestinal lumen and due to the inability of the immune system to eradicate the agent[6,16].

Patients treated with metronidazole relapse more than those treated with vancomycin (21.2 *versus* 16.7%, respectively)[15,16]. Therefore, in cases of the recurrence cases, the same treatment may be used or vancomycin be preferred[16].

The main risk factors for relapse are advanced age, chronic kidney disease, previous episodes of CD, leukocytes ($\geq 15 \times 10^9/\mu L$) and the use of systemic antibiotics concurrent to treatment for CD[16].

FINAL CONSIDERATIONS

CD is one of the major complications related to healthcare and is intrinsically related to the use of antibiotics. It is easily disseminated at hospitals due to spore formation. Unfortunately, elderly patients develop the disease more easily, with worse prognosis and more chances for relapse. The diagnosis is based on diarrhea with positive toxin in the stools test. Metronidazole is the main drug for treating this infection. New drugs that are more efficient and lead to lower recurrence rate have been launched.

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