

Clostridium Difficile infection and Antibiotic –Associated Colitis

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ABSTRACT: Hospitalized patients receiving β lactam antibiotics develop diarrhea and higher rates reported in those receiving clindamycin. *Clostridium difficile* is recognized as a cause of antibiotic associated diarrhea, colitis and pseudomembranous colitis. Contributory factors include: advance age, severity of underlying illness. *C. difficile* has the ability to temporarily colonize newborn humans and calves, suggests that the gastrointestinal tracts of young animals may be a major reservoir. *C. difficile* is recognized enteric pathogen in animals including companion animals and food animals. Clinical features include: asymptomatic carriage in neonates to a fulminant, relapsing, and occasionally fatal colitis. Initial therapy for pseudomembranous colitis includes discontinuation of offending antibiotic regimen, fluid replacement and electrolyte losses. Mild to moderate infection with metronidazole, severe to recurrent cases with vancomycin. Therapy with probiotic such as *Saccharomyces boulardii* is beneficial. Antibiotic misuse in humans and production animals must be addressed.

KEY WORDS: *Clostridium difficile*, Antibiotic, Colitis, and Diarrhea

I. INTRODUCTION

Antibiotic associated diarrhea and colitis is most common complications of antibiotic therapy. Attack rates vary depending on the antimicrobial agent used, the epidemiologic setting, and the host. Overall attack rates antibiotic-associated diarrhea in hospitals range from 3.2% to 29% [1]. Almost 15% of hospitalized patients receiving β -lactam antibiotics develop diarrhea and rates for those receiving clindamycin range from 10% to 20% [2]. *Clostridium difficile* is now recognized as a frequent cause of antibiotic associated diarrhea and colitis, and the incidence of *C. difficile* associated diarrhea seems to be increasing [3]. It is implicated in 20% to 30% of patients with antibiotic-associated diarrhea, in 50% to 75% of those with antibiotic-associated colitis [4]. Literature from the mid-1970s emphasized that attack rates for diarrhea and pseudomembranous colitis associated with the use of individual antibiotics. Several studies rates of clindamycin and lincomycin associated diarrhea ranging from 7% to 21% and rates of ampicillin-associated diarrhea from 4% to 17% [5]. In the late 1970s, a series of investigations established toxigenic *C. difficile* as the cause of pseudomembranous colitis [6]. The reported incidence of *C. difficile* in acute-care hospital increased from 30 to 40 cases per 100,000 population in 1990s to 84 per 100,000 by 2005 [7]. Although colonization with toxigenic *C. difficile* occurs frequently among residents of some extended-care and rehabilitation facilities symptomatic disease develops in only a minority of infected patients [8]. Animal studies found that intracecal materials transferred the disease from affected hamsters to healthy ones that both the cultures of *Clostridia* and their cell-free supernatants produced this disease and this activity was neutralized by gas gangrene antiserum [9]. Ritkin and associates reported that stool filtrates from the humans with pseudomembranous colitis were lethal for hamsters; caused edema, hemorrhage, and increased vascular permeability in rabbit skin and possessed cytotoxic activity that was neutralized by *Clostridium sordelli* antitoxin [10]. Emergence of strains causing *C. difficile* infection in the community (CA-CDI) is becoming more commonplace [11]. Unlike health care infections, CA-CDI is associated with younger, healthy people, often without prior exposure to antimicrobials or contact with hospitalized patients [12]. The rates of human infection CDI have increased dramatically and *C. difficile* is recovered from Australian production animals [13,14]. The paper reviews pathophysiology, clinical features, diagnosis and therapy of antibiotic associated colitis.

II. PATHOPHYSIOLOGY

C. difficile most often causes a nonspecific colitis. However, especially in more severe cases, one may see the distinct macroscopic appearances of pseudomembranous colitis. The colonic mucosa is studded with adherent, raised, white and yellowish plaques. Initially, these lesions are small and discrete and are easily dislodged; the intervening mucosa may be inflamed and covered with mucus, but often it appears entirely normal [15]. With progression of disease, pseudomembranous plaques may enlarge and coalesce. Pseudomembranes can exist throughout the entire colon, but they are usually most pronounced in the recto sigmoid

colon; rarely does the disease progress proximal to the ileocecal valve [15]. Histological criteria for pseudomembranous colitis and method for grading lesions have been described. The principal features are inflamed mucosa with a neutrophil predominance and mucin distended glands. Attached pseudomembranous are composed of a loose network of mucin, neutrophils, fibrin, and nuclear debris [15].

III. RESERVOIRS OF INFECTION

Researchers have documented the prevalence of *C.difficile* (or one of its toxins) in 15% to 70% of neonates [16]. Despite the presence of toxin-producing organisms in this population, the prevalence of *C.difficile* colitis remain low [17]. Enhancement of chemotactic responses of granulocytes to toxin A in older persons and the absence of high-affinity receptors for toxin A in neonates (in a rabbit model) have been cited as possible reasons for this age-dependent susceptibility [18,19]. The ability of *C.difficile* to temporarily colonize newborn humans, hamsters, and calves suggests that the gastrointestinal tract of very young mammals may be a major reservoir [19,20]. In healthy adults, intestinal carriage rates of toxigenic *C.difficile* are typically 3% or less and not greater than 8%. Asymptomatic intestinal carriage rates are higher (-approximately 20%) among hospitalized adults, particularly those who have received antibiotics [21,22].

C.difficile is a recognized enteric pathogen in a variety of animals including companion animals e.g. Cats dogs, horses, and food animals (cattle, sheep, goats, pigs) [23]. In Australia *C.difficile* has been isolated from piglets, sheep, lambs, horses, cats, dogs and cattle with the highest prevalence in neonatal animals due to a lack of established gut flora at birth. For this reason predisposing antibiotics may not be required for development of CDI in young animals although there is anecdotal evidence in Australia of routine use of extended-spectrum cephalosporins in production animals. This is particularly concerning in the pork industry where gross contamination of facilities with *C.difficile* spores is commonplace with *C.difficile* [24]. Several meat products, seafood, ready-to eat salad leaves and vegetables are also contaminated with *C.difficile*, predominantly ribotype 078-like strains [16]. Contamination may occur through spillage of gut contents at slaughter or direct contamination by food handlers during dressing or retailing. Environmental contamination may also play a role. *C.difficile* spores survive in treated piggery effluent, the byproducts of which are often applied to agriculture land, used in retail compost manufacture, or recycled within swine facility [25].

Outside Australia, the increasing prevalence of PCR ribotype 078 in humans, food production animals and food products suggest potential zoonotic transmission. In Netherlands, where infections with ribotype 078 increased more than fourfold from 2005 to 2008, patients infected with this ribotype were younger and acquired *C.difficile* in the community more frequently particularly if they lived in rural pig producing areas [26]. In the USA, the prevalence of ribotype 078 infections in humans increased from 0.02% to 1.3% (pre 2001 to 2006) and ribotype 078 is increasingly associated with CA-CDI. These strains are indistinguishable or very closely related to animal ribotype 078 strains by PFGE analysis [27]. Ribotype 078 strains from Dutch humans and pigs are indistinguishable by MIVA subtyping [28]. Transmission from humans to animals may occur. *C.difficile* can be isolated from the feces of hospital pet therapy dogs that had prior negative culture for *C.difficile*. These dogs were > 2 times likely to be colonized with *C.difficile* than dog's not visiting hospital [29].

IV. EXASPERATE AGENTS

Almost all antibiotic classes have been associated with the disease, reports of large clinical series' most commonly implicate clindamycin, penicillins, cephalosporins and more recently fluoroquinolones [30]. Several noteworthy studies primarily implicated clindamycin and ampicillin in the 1970s [31]. In the most recent outbreaks associated with NAPI/027 (North American pulsed field type 1, ribotype 027) which is resistant to fluoroquinolones, fluoroquinolones are clearly identified as a risk factor for clinical disease [32]. Third generation cephalosporins have been implicated in particular and seem to predispose to *C.difficile*-associated disease more commonly than do the narrow spectrum penicillins or β -lactam-stable penicillin [33]. A number of antineoplastic agents particularly those with modest antibacterial activity, have been associated with *C.difficile* diarrheal disease, including doxorubicin, cisplatin, cyclophosphamide and others [34].

V. CONTRIBUTORY FACTORS

Antimicrobials use is the primary factor in the disease, but certain host and environmental factors also predispose to *C.difficile*-associated disease. In a comprehensive prospective study of risk factors in hospitalized patients, McFarland and associates [35], identified advanced age and severity of underlying illness as factors associated with increased risk of *C.difficile* carriage and diarrhea and found that agents that alter normal intestinal motility—specifically enemas and gastrointestinal stimulants, as well as stool softener—also contribute to the risk of *C.difficile* associated diarrhea [35]. Given the tendency of this disease to affect ill elderly patients who receive antibiotics, it is no surprise that patients with *C.difficile* are also at risk of colonization by vancomycin resistant enterococci [36]. Other investigators have reported that critically ill burn patients, uremic patients, patients with hematologic malignancies, and those undergoing gastrointestinal surgery are at high risk of

C. difficile diarrhea and colitis [37-40]. Emerging evidence suggests that immunologic susceptibility may play a critical role in *C. difficile*-associated infection. Host immunoglobulin G responses have been shown to protect against symptomatic disease and relapse [41,42]. Human deficiency virus (HIV) infection per se does not appear to predispose to *C. difficile* colonization and specific risk factors for *C. difficile*-associated disease among HIV-infected patients appear to be similar to those HIV seronegative persons [43,44]. In recent years CA-CDI has become more common, and in nearly one fourth of such cases, no traditional risk factor are identified [45]

VI. CLINICAL FEATURES

Infection with toxigenic *C. difficile* causes a spectrum of disease ranging from asymptomatic carriage (particularly in neonates) to a fulminant, relapsing, and occasionally fatal colitis. When *C. difficile* produces clinical disease, the onset of signs and symptoms typically occurs after 3 to 5 days of antibacterial treatment, but diarrhea may develop as early as first of therapy or as late as 10 weeks after cessation of therapy [46]. *C. difficile* diarrhea may be brief and self-limited or it may be cholera like, resulting in more than 20 stools per day [2]. Accompanying findings often include fever (30% to 50% of patients), leukocytosis (50% to 60%) and abdominal pain or cramping (20% to 33%) [47,48,47]. The mean peripheral leukocyte count of patients with *C. difficile*-associated diarrhea typically 15,000 to 16,000/mm³ in one series. *C. difficile* infection was noted in 25% of patients with white cell counts of greater than of greater than 35,000/mm³ who did not have hematologic malignancy [49]. Nausea, malaise, anorexia, hypoalbuminemia, occult colonic bleeding, and dehydration have also been reported [2]. Infrequently, *C. difficile* colitis manifests without diarrhea as an acute abdominal syndrome or toxic megacolon [50]. In one report, 5 of 97 patients with *C. difficile* disease (only 1 of whom had profuse diarrhea) presented initially with marked leukocytosis (white cell count >25,000/mm³) and right lower quadrant peritoneal signs mimicking those of acute peritonitis [51]. Toxic megacolon is suggested by acute dilatation of colon to a diameter greater than 6 cm, associated systemic toxicity, and the absence of mechanical obstruction. It carries a high mortality rate (64% in one series of 11 patients) [52]. Other intra-abdominal complications include colonic perforation, transverse volvulus, protein-losing enteropathy, and recurrent *C. difficile*-associated diarrhea, the last occurring in approximately 20% of patients [53,54,55]. Extra intestinal manifestation occur more rarely and include bacteremia, often with concurrent isolation of other constituent organisms in the bowel flora, splenic abscess, and osteomyelitis [56,57,58]. In addition, multiple reports have described reactive arthritis or tenosynovitis or both [59,60].

Consideration in the differential diagnosis of antibiotic-associated colitis include diarrhea caused by other enteric pathogens [especially *Salmonella*], adverse reactions to other medications, ischemic colitis, inflammatory bowel disease, and intra-abdominal sepsis [50].

VII. DIAGNOSTIC METHODS

C. difficile-associated disease should be suspected in patients with diarrhea who have received antibiotics within the past two months or whose diarrhea begins 72 hours after hospitalization [50]. In most instances, toxin testing or *C. difficile* culture of a single stool specimen effectively establishes the diagnosis; however, repeat testing or endoscopy, or both may be necessary

C. difficile toxin detection

The most widely used means of diagnosing *C. difficile*-associated diarrhea and colitis clinically detection of *C. difficile* toxin in stool specimens. The cytotoxicity assay has been considered the gold standard for diagnosis, but most clinical laboratories use various enzyme immunoassays, which perform reasonably well compared with cytotoxicity assays [6]. Stool filtrate are incubated with mammalian tissue culture cell line with and without toxin-neutralizing antibody. If toxicity activity, usually manifested as surrounding of cultured cells, is noted in the well with stool filtrate and toxin-neutralizing antibody, the test for the specimen is considered to be positive [6]. If used in appropriate clinical setting, this test is both sensitive and specific; more than 90% of patients with pseudomembranous colitis have cytotoxicity in their stool detected by this assay [61].

C. difficile toxin A or B can be detected by enzyme-linked immunosorbent assay kits, available commercial kits, their performance characteristics have been studied extensively [62]. In general, they are rapid, relatively inexpensive, and specific, but lack the sensitivity of the technically more cumbersome cytotoxin assays. If measured against strictest diagnostic criteria that include clinical diarrhea, along with cytotoxin assay and culture results, their sensitivity ranges from 63% to 94% with a specificity of 75% to 100% [63].

C. difficile detection by culture

Anaerobic culture for *C. difficile*, the most sensitive test in many laboratories, remains essential for epidemiologic studies of outbreaks. However, cost and convenience issues have driven many hospital laboratories to replace routine cultures with more rapid and less expensive enzyme-linked immunosorbent assay based assays, sometimes in combination with cytotoxin assays. Most clinical microbiology laboratories are not

equipped to distinguish between nonpathogenic, on toxigenic strains, and testing schemes that rely solely on *C.difficile* cultures yield a significant number of false-positive results (In some hospitals, 20% to 25% of *C.difficile* isolates are non-toxigenic [64]. Testing for stool toxin simultaneously or using in vitro toxin production assays may help to resolve this problem [63].

Endoscopy for detection of pseudomembrane

The detection of exudative plaques or pseudo membranes on colonic mucosa establishes the diagnosis of pseudomembranous colitis. The pathognomonic lesions is characteristically raised, yellowish, and usually 2 to 10 mm in diameter with “skip” of normal mucosa, but in severe disease, lesions may coalesce to form plaques [50]. At least 90% of patients with pseudomembranous colitis demonstrate either *C.difficile* or its toxin in stool samples [48]. Because of its cost, risk to the patient, and availability of other diagnostic tests, endoscopy is usually reserved for special situations [50].

VIII. THERAPY

Treatment of choice in *Clostridium difficile*-associated diarrhea and colitis for adults include: Initial therapy for pseudomembranous colitis should commence with discontinuation of the offending antibiotics regimen, if possible, and replacement of fluid and electrolyte losses. In a prospective treatment trial the diarrhea resolved before initiation of therapy in 23% of 149 patients, but most patients require specific antibacterial therapy [65]. Therapy of *Clostridium difficile*-associated diarrhea and colitis for adults begins with replace fluid and electrolytes, if clinical situations allows, discontinue offending antibiotic, and avoid antiperistaltic agents plus for mild to moderate infection metronidazole 500 mg orally 3 times daily for 10 to 14 days. In severe infection or unresponsiveness to or intolerance to metronidazole, vancomycin 125 mg daily 4 times daily for 10 to 14 days. For second recurrence, vancomycin in tapered and pulsed doses a) 125 mg 4 times daily for 14 days, b) 125 mg orally twice daily for 7 days, c) 125 mg once daily for 7 days, d) 125 mg orally once every 2 days for 8 days) 125 mg once every 3 days for 15 days [15]. Other options for recurrent infection include: Vancomycin followed by rifamiximin, 400 mg orally 2 times daily for 14 days. Therapy with probiotic such as *Sacchromyces boulradii* [15].

Antiperistaltic agents, such as loperamide and diphenoxylate hydrochloride with atropine should be avoided. There is little evidence that such agents lead to symptomatic improvement, and in one study predating the discovery of *Clostridium difficile* as a pathogen, diarrhea was actually more common among those receiving diphenoxylate-atropine plus lincomycin than those receiving placebo plus lincomycin [66]. Several anecdotes and case series have associated the use of diphenoxylate and of loperamide and other anti-peristaltic agents with the development of toxic mega colon in patients with *C.difficile* disease or pseudomembranous colitis [51].

IX. CONCLUSION

Diarrhea and colitis is most common complications of antibiotic therapy. *C.difficile* causes diarrhea in humans and animals. Toxigenic *C.difficile* is also cause of pseudomembranous colitis. CA-CDI strains causing infection in the community is also common. Antimicrobial use in humans and animals must be addressed.

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