Treatment of *Clostridium difficile*-associated disease: old therapies and new strategies

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Clostridium difficile*-associated disease (CDAD) causes substantial morbidity and mortality. The pathogenesis is multifactorial, involving altered bowel flora, production of toxins, and impaired host immunity, often in a nosocomial setting. Current guidelines recommend treatment with metronidazole; vancomycin is a second-line agent because of its potential effect on the hospital environment. We present the data that led to these recommendations and explore other therapeutic options, including antimicrobials, antibody to toxin A, probiotics, and vaccines. Treatment of CDAD has increasingly been associated with failure and recurrence. Recurrent disease may reflect relapse of infection due to the original infecting organism or infection by a new strain. Poor antibody responses to *C difficile* toxins have a permissive role in recurrent infection. Hospital infection control and pertinent use of antibiotics can limit the spread of CDAD. A vaccine directed against *C difficile* toxin may eventually offer a solution to the CDAD problem.

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Introduction

Clostridium difficile is the leading identified cause of nosocomial diarrhoea associated with antibiotic therapy (figure). Occurrence of diarrhoea in hospitalised inpatients who receive antibiotics ranges from 3% to 29%.[1] *C difficile* has been implicated as the causative organism in 10–25% of patients with antibiotic-associated diarrhoea, 50–75% of those with antibiotic-associated colitis, and 90–100% of those with antibiotic-associated pseudomembranous colitis.[2,3] Mortality of *C difficile*-associated disease (CDAD) ranges from 6% to 30% when pseudomembranous colitis is shown to be present,[4–7] and is substantial even in the absence of colitis. The incidence of CDAD has increased in the past decade, with a 10-fold increase reported in Quebec,[7] as has the proportion of patients who have severe, refractory, or recurrent disease.[7–9] Hospital costs attributable to this condition in the USA[10] and UK[11] exceed US$4000 per case.

History of *C difficile* colitis

First described in the 1950s, pseudomembranous enterocolitis was attributed either to *Staphylococcus aureus*, an organism that had become prevalent in hospital inpatients who had received antibiotics,[12–16] or to *Candida albicans*.[17] In 1974, a prospective study of 200 patients treated with clindamycin detected diarrhoea in 21% and pseudomembranous colitis in 10%.[18] A toxin produced by a *Clostridium* species was proposed as the cause of clindamycin-induced ileocaecitis in hamsters in 1977;[19] later this toxin was isolated from patients' stool samples, with evidence and counter-evidence presented for *C difficile* and *Clostridium sordellii* as causative organisms.[20–22] However, by 1978, *C difficile* had been clearly identified as the causal agent of antibiotic-associated colitis.[23]

The initial treatment for CDAD was oral vancomycin. In the early 1980s, metronidazole was also shown to be effective, perhaps equally so, and a strong preference to avoid the use of vancomycin in hospital inpatients,

reinforced by several sets of therapeutic recommendations,[24–26] has led to increasing reliance on metronidazole. In 1997, the American Gastroenterology Association published recommendations for treating CDAD that included discontinuation of antibiotics, supportive non-specific therapy, and addition of metronidazole for those who failed to respond within 2–3 days.[27] Oral vancomycin was recommended for the following categories of patients: those who were critically ill, unable to tolerate metronidazole, pregnant, or under the age of 10 years; those who failed initial therapy with metronidazole; or those whose infecting organism proved to be metronidazole resistant. The past few years have witnessed an increase in the failure rate of antimicrobial therapy.[8,9,28] Some patients simply fail to respond to conventional therapy, and others promptly relapse after discontinuation of treatment. The

Figure: Coloured transmission electron micrograph of *Clostridium difficile* forming an endospore (red)

Cochrane database reports only nine well-designed randomised trials that have assessed treatments for CDAD.[29] The purpose of this review is to examine current recommendations for therapy, to assess other possible modes of treatment, and to cite areas for future investigation.

Microbiology and epidemiology

Hall and O'Toole[30] first described *C difficile* in 1935 as part of the normal flora of neonates. The organism is a Gram-positive, spore-forming rod that is an obligate anaerobe. It is relatively large (2–17 µm in length) and fast growing; CCFA medium (consisting of cycloserine, cefoxitin, and fructose agar in an egg-yolk agar base) is highly selective for its growth. Toxin A, toxin B, and the binary toxin of *C difficile* may contribute to CDAD. Toxin A causes fluid secretion and intestinal inflammation when injected into rodent intestine[31] and is a chemoattractant for neutrophils in vitro.[32] Toxins A and B both activate the release of cytokines from monocytes.[33] It is unclear whether the binary toxin is pathogenic; however, a recent study has shown a trend toward more severe disease in patients who carry the strain of *C difficile* that produces binary toxin.[34]

C difficile can be cultured from the stool of 3% of healthy adults.[35] Stool carriage of *C difficile* reaches 16–35% in hospital inpatients, with the percentage proportional to the duration of hospital stay and increasing with exposure to antibiotics.[36–38] Newly admitted patients who are already colonised seem to be an important source of contagion in hospitals.[38] CDAD may also occur in outpatients, although incidence is low.[39,40]

Pathogenesis and immunity

The pathogenesis of CDAD is complex and incompletely understood. The congruence of debilitating diseases and antibiotic therapy (sometimes chemotherapy) in hospital inpatients is thought to alter the bacterial flora of the colon, thus creating conditions that favour new acquisition and proliferation of *C difficile*.[3,41] Individuals who acquire *C difficile* may be colonised or develop disease, and the immune status of the host is an important determinant of the outcome. Patients with more severe underlying illnesses are more likely to develop CDAD.[42] People who carry *C difficile* without developing colitis have higher concentrations of serum antibody to toxin A than do symptomatic patients,[43,44] and are less likely to develop clinical disease.[45] For patients who develop CDAD, higher concentrations of anti-toxin A antibody are associated with a shorter duration of illness and a decreased risk of recurrence.[5,46]

Stopping the offending antibiotic

In three early studies,[4,47,48] 15–23% of patients with CDAD had spontaneous resolution of symptoms within 48–72 h of stopping the antibiotic that was associated with the disease episode and without specific antimicrobial therapy. Continuation of systemic antibiotics has been associated with refractoriness to treatment.[49] However, one cannot predict which patients will clear the infection spontaneously, and to discontinue antibiotics is often not feasible. In addition, the time between the onset of symptoms and the confirmation of CDAD may be a few days in the usual clinical scenario, and further delay in starting treatment in symptomatic patients may lead to clinical deterioration. Thus, in theory, although discontinuing the antibiotics and observing the patient might be effective in a small proportion of patients, it is difficult to apply this approach in practice.

Specific therapy

Vancomycin

Oral vancomycin was used to treat so-called staphylococcal enterocolitis and clindamycin-associated diarrhoea before the discovery that *C difficile* was responsible for the disease.[2,50,51] Recognition of this organism's role was followed by additional studies of vancomycin for treatment.[48,52–55] Between 1977 and 1980, most physicians prescribed oral vancomycin for 7–14 days to treat confirmed cases of CDAD,[48] with clinical resolution observed in almost 90% of treated patients.[48,53] Doses of vancomycin ranging from 125 mg to 500 mg four times daily were found to be equally effective.[55] Subsequent studies showed the percentage of patients cured by oral vancomycin to be 86–100%.[2,4,47,56–58] The table summarises treatment failures and recurrences in published series of cases.

In vitro, *C difficile* is susceptible to vancomycin; the reported minimum inhibitory concentration (MIC) required to inhibit 90% of strains (MIC_{90}) is 0·75–2·0 µg/mL.[66–72] A recent study from Madrid found that 3% of *C difficile* isolates had intermediate resistance to vancomycin (MIC 4–16 µg/mL), but clinical correlation was not provided.[73] Orally administered vancomycin has limited absorption and has a stool concentration of up to 3100 µg/g,[52] suggesting that the resistance reported to date is not clinically important.

Metronidazole

In 1982, Cherry and colleagues[59] described 13 patients with CDAD who were treated with 1·5–2·0 g oral metronidazole daily for 7–10 days. All 13 patients responded, although two (15%) had recurrent disease. Soon thereafter, a randomised trial in 92 patients compared 250 mg oral metronidazole four times daily with 500 mg oral vancomycin four times daily;[47] treatment was given for 10 days. Responses to treatment (88% for vancomycin, 90% for metronidazole) and recurrence within a 21-day follow-up period (12% for vancomycin, 5% for metronidazole) were similar.

The apparent equivalence of these two drugs and continuing concern over the selection of vancomycin-

resistant bacteria, especially in hospitals, led the US Centers for Disease Control and Prevention to recommend that metronidazole be used as first-line therapy for CDAD.[27,74,75] One study reported a 98% response with 7% recurrences in 632 CDAD patients treated with metronidazole.[4] A response of 90–98% with the use of metronidazole has also been noted in other studies.[47,60] However, Nair and co-workers[49] described a small series of patients treated either with vancomycin or metronidazole in which 25% failed to respond to a 2-week course of treatment, and 26% had a recurrence within 3 months. Our experience in a large observational study was similar.[8] Infectious disease physicians in North America have also noted a recent increase in the number of refractory and recurrent disease episodes.[28]

In vitro, the MIC$_{90}$ of metronidazole for *C difficile* ranges from 0·20 μg/mL to 2·0 μg/mL (median <1 μg/mL).[66,68–72] Resistance has been reported,[76,77] including an isolate from Hong Kong[68] with an MIC of 64 μg/mL and in 6/198 (3%) French isolates[70] with an MIC of 8–32 μg/mL. Using disk diffusion, Pelaez and colleagues[73] found that 26/415 (6·3%) Spanish isolates from patients with their first episode of CDAD had an MIC of 32 μg/mL or more. However, no clinical correlation has been provided. Our results[8] and those of Sanchez and colleagues[78] have shown that the metronidazole susceptibility of *C difficile* in patients with clinical treatment failure was similar to those who had clinically responded to metronidazole therapy. Thus, whether metronidazole resistance has an important role in treatment failure and recurrence is unclear.

After ingestion by healthy volunteers, metronidazole is completely absorbed from the gastrointestinal tract and is undetectable in faeces.[79,80] However, concentration of this drug in faeces is significantly higher when stools are watery or semi-formed than when they are solid (p<0·05), generally exceeding the MIC for *C difficile* while diarrhoea is present (mean concentration of metronidazole 9·3 μg/g, range 0·8–24).[81] This occurence may result from increased gastrointestinal transit time leading to incomplete absorption or seepage of the drug across the inflamed colonic mucosa.[81] In semi-formed stool, drug concentration is somewhat lower (mean 3·3 μg/g, range 0·5–10·4) and are almost undetectable during convalescence (mean 1·2 μg/g, range 0–10·2). A correlation between faecal metronidazole concentrations and clinical outcome has not been reported, but if the high MICs of metronidazole for *C difficile* are correct, resistance may become clinically relevant, especially because concentrations are so low after diarrhoea has subsided.

Very limited data suggest that parenteral metronidazole might also be useful in treating CDAD. In three patients who received intravenous metronidazole,[81] faecal concentrations ranged from 6·3 μg/g to 24 μg/g of stool during acute illness but were significantly lower in formed stool (p<0·05). A retrospective review of

Studies	Treatment failures [n/total (%)]	Recurrences [n/total (%)]	Duration of follow up (days)	Percentage failure plus recurrence
Metronidazole				
Cherry et al, 1982[59]	0/13	2/13 (15%)	30	15%
Teasley et al, 1983[47]	2/42 (5%)	2/39 (5%)	21	10%
Olson et al, 1994[4]	14/632 (2%)	39/632 (6%)	30	8%
Wenisch et al, 1996[60]	2/31 (6%)	5/31 (16%)	30	22%
Kyne et al, 2001[5]	..	22/44 (50%)	60	..
Fernandez et al, 2004[61]	38/99 (38%)	..	..	..
Musher et al, 2005[8]	46/207 (22%)	58/207 (28%)	90	50%
Pepin et al, 2005[9]	178/1123 (16%)	243/845 (29%)	60	45%
Vancomycin				
Bartlett et al, 1980[62]	3/79 (4%)	11/79 (14%)	30	18%
Silva et al, 1981[53]	0/16	2/16 (13%)	42	13%
Teasley et al, 1983[47]	0/52	6/51 (12%)	21	12%
Bartlett, 1984[48]	6/189 (3%)	46/189 (24%)	25	27%
Young et al, 1985[56]	8/42 (19%)	11/30 (37%)	30	56%
Dudley et al, 1986[57]	0/15	3/15 (20%)	60	20%
de Lalla et al, 1989[63]	2/25 (8%)	3/25 (12%)	30	20%
Fekety et al, 1989[55]	0/46	9/46 (20%)	42	20%
de Lalla et al, 1992[58]	0/20	4/20 (20%)	30	20%
Olson et al, 1994[4]	1/122 (1%)	12/122 (10%)	30	11%
Wenisch et al, 1996[60]	2/31 (6%)	5/31 (16%)	30	22%
Pepin et al, 2005[9]	..	31/112 (28%)	60	..
Metronidazole and vancomycin				
McFarland et al, 1994[64]	8/33 (24%)	8/33 (24%)	60	48%
Nair et al, 1998[49]	9/36 (25%)	7/36 (19%)	90	44%
Noren et al, 2004[65]	..	68/267 (25%)	60	..

Table: Studies of antibiotic treatment in CDAD: treatment failure and recurrences

10 patients with CDAD who were initially treated with intravenous metronidazole for a mean of 4 days showed clinical improvement in nine patients.[82] We have used this approach successfully in patients who have toxic megacolon (SA, RJH, DMM; unpublished data). As a note of caution, there is a case report that described failure of intravenous metronidazole in the treatment of pseudomembranous colitis.[83] To our knowledge, no trial has compared oral with intravenous metronidazole for the treatment of CDAD.

Bacitracin

Bacitracin was successfully used to treat isolated cases of CDAD in the 1980s,[84] and was subsequently compared with vancomycin in two randomised clinical trials.[56,57] There was no difference between the drugs in the clinical response, which ranged from 76% to 100%. On completion of therapy, 55% of patients receiving bacitracin and 14% of those receiving vancomycin still had *C difficile* toxin in the stool (p<0·05), but this result did not affect the number of clinical recurrences.[57]

Teicoplanin and fusidic acid

Teicoplanin and fusidic acid, neither of which is available in the USA, have been shown to have similar efficacy to oral vancomycin[58,63] and metronidazole.[85] A European study[60] prospectively compared oral vancomycin, metronidazole, teicoplanin, and fusidic acid in 119 patients with CDAD and found that 93–96% were clinically cured for all regimens. However,

treatment with fusidic acid was associated with a significantly higher recurrence (28%, p=0·04) and a higher proportion of adverse events (31% had gastrointestinal discomfort, p=0·001).

Nitazoxanide

Approved for treating protozoan and helminthic infections[86–89] in the USA in December, 2003, nitazoxanide has already been used to treat 5 million people around the world for these diseases. This drug blocks anaerobic metabolic pathways of microorganisms and is effective against *C difficile* in vitro (MIC_{90} 0·06–0·5 µg/mL).[90,91] In vivo, nitazoxanide prevents colitis after challenge of hamsters with *C difficile*,[90] although it has not been shown to treat established disease. In human beings, approximately two-thirds of the oral dose is excreted in faeces as an active metabolite called tizoxanide, which has an MIC_{90} of 0·06 µg/mL for *C difficile*.[91,92] This metabolite has been found at a concentration of 200 µg/mL in human bile after a 1000 mg oral dose (information on file with the US Food and Drug Administration [FDA]) and thus high intraluminal concentrations can be achieved. An open-label, prospective, compassionate-use study in our hospital has shown that treatment with nitazoxanide cured 75% of patients who had failed metronidazole therapy, although one-third of these later relapsed (SA, RJH, DMM; unpublished data); and a double-blind, controlled trial comparing these two drugs is currently underway.

Non-antimicrobial treatments

Antiperistaltic agents—eg, loperamide and diphenoxylate—should be avoided in CDAD. Several case reports have linked the use of antiperistaltic agents in patients with CDAD with the development of toxic megacolon,[93,94] probably because they delay excretion of toxin. Pooled human immunoglobulin (200–500 mg/kg) has been used with variable success to treat refractory CDAD in individual patients.[95,96] Anti-*C difficile* bovine immunoglobulin neutralises the effects of toxin B in the cell cytotoxicity assay, and has been used to treat and prevent *C difficile* colitis in rodents.[97,98] Use of a monoclonal antibody to toxin A has shown promising results in animals, and phase II studies in human beings are currently in progress.[99] The anion exchange resins colestipol and cholestyramine bind the toxin produced by *C difficile*, but lack clinical efficacy,[100–102] and their potential is further compromised by the possibility that they also bind drugs that are used to treat the disease, such as vancomycin.[103] Short courses of intravenous methylprednisolone have also been used to effectively treat CDAD in a few patients,[104] although because steroids have only been used on a case report basis, formal recommendations cannot be made.

The panel summarises treatment recommendations.

Panel: Treatment recommendations

- Stop treatment with the offending antibiotic, if possible.
- Replete fluid and electrolytes.
- Do not use antimotility agents.
- If specific treatment is required, use metronidazole 500 mg orally every 6–8 hours for 7–10 days. Vancomycin at a dose of 125 mg orally every 6 hours is a second-line alternate agent. If the patient cannot tolerate the drug orally, use intravenous metronidazole, but this should be switched to oral therapy once able to tolerate it. In the case of ileus or toxic megacolon, use intravenous metronidazole, perhaps adding vancomycin retention enemas in a dose of 500 mg mixed in 100 mL normal saline.
- Avoid using vancomycin unless metronidazole seems ineffective, the patient is pregnant or allergic to metronidazole, or true resistance is shown.
- In case of recurrence, re-treat with the agent that had been used to treat the initial episode of CDAD, usually metronidazole.
- In case of multiple recurrences or refractory disease, consider the use of probiotics, immunoglobulin, or steroids.
- In all cases, strict contact isolation of the patient is essential in controlling the spread of the disease to other patients.
- Do not treat symptom-free carriers.

CDAD in patients with HIV infection

Patients with HIV/AIDS are as likely to respond to therapy as other patients,[105,106] a finding that might be thought of as surprising in light of the putative role for humoral immunity (see below). Our own observations suggest that patients who have AIDS and develop CDAD, many of whom are younger adults, tend to respond better to treatment than do debilitated, elderly individuals with CDAD.

Recurrences

CDAD recurs after treatment in 8–50% of cases,[4,47–49,53,56–60,62] with recent reports of increases in both recurrent and refractory disease.[7–9,28] A single recurrence tends to be followed by repeated episodes, perhaps in as many as 65% of patients.[5,64,107] New exposure to antibiotics, especially multiple antibiotics, is an important risk factor for recurrence,[49,107] as is age older than 65 years,[5,9] severity of underlying illness,[5] a low serum albumin concentration (<2·5 g/dL),[49,61] stay in an intensive care unit,[61] and hospital stay of 16–30 days.[9]

It was initially assumed that infection recurred because *C difficile* sporulated during treatment and then germinated once treatment was completed. However, by use of serotyping, PCR ribotyping, or chromosomal restriction endonuclease analysis, several reports have implicated new strains of *C difficile* in 10–50% of

recurrent infections.[65,108–111] This finding suggests that nosocomial re-acquisition of hospital-associated strains is partly responsible for recurrent CDAD. Recurrence may also reflect a poor immune response during the initial infection. Antibody to *C difficile* toxin A can be detected in most patients by 3 days after colonisation occurs.[5,44] A higher concentration of anti-toxin antibody in the initial episode of CDAD is associated with a decreased risk of recurrence.[5,46]

Treatment of recurrent disease

Various strategies have been proposed to treat recurrent CDAD. Longer courses of therapy are commonly given, although this approach would neither be needed to treat reinfection nor expected to be effective against sporulating organisms. Vancomycin has been given in a pulsed dose or a tapered regimen, on the basis that drugs given every few days or in a decreasing dose would allow the *C difficile* spores to germinate and thus be susceptible to the antibiotic.[112] A combination of vancomycin and rifampicin has also been reported as effective treatment in a few cases.[113] Our experience has been that repeated courses of metronidazole or vancomycin seem to have similar responses of 70–78% with further recurrences in an additional 25%.[8] The issue of recurrent CDAD remains a vexing problem with no satisfactory resolution at present.

With a better understanding of the pathogenesis of CDAD, treatment has been directed either to restoring a normal colonic ecosystem (which is presumably inimical to the growth of *C difficile*) or to bolstering the immune response. Stool infusions, in an effort to repopulate the colon with normal colonic flora, have been reported effective in refractory cases.[114,115] Such therapy lacks aesthetic appeal, not to mention the risk of transferring communicable diseases.

There has also been growing interest in probiotics—the use of non-pathogenic organisms to repopulate the colonic microflora, and thus, presumably, restrict the growth of toxigenic *C difficile*. Agents that have been studied include a non-toxigenic strain of *C difficile*,[116] *Saccharomyces boulardii*, and *Lactobacillus* spp.[117,118] McFarland and colleagues[64] studied the addition of *S boulardii* to vancomycin or metronidazole in a prospective, double-blind study; in 60 patients with recurrent CDAD, there were fewer recurrences in those patients who received *S boulardii* (35% *vs* 65%, p=0·04). However, treatment with *S boulardii* did not decrease recurrences in patients treated for their first episode of CDAD. A placebo-controlled pilot study noted a trend towards a decreased incidence of CDAD in hospital inpatients who were given *Lactobacillus* and *Bifidobacterium* spp at the time antibiotic therapy was started, although the results did not reach significance.[119] Although a meta-analysis found that odds ratios from available randomised studies favoured

a role for probiotics over placebo,[120] the US FDA was not convinced that the data supported efficacy of *S boulardii*.

Recurrent CDAD has been treated with some success with intravenous immunoglobulin.[96,121,122] No prospective clinical trial has been reported, and it is not known whether the commercial globulin preparations contain antibody to *C difficile* toxin, or whether antibody to some other antigen is responsible for the putative beneficial effect. On the basis that recurrence is more likely in individuals who lack anti-toxin A antibody, infusion of such antibody or vaccination with a toxoid might be beneficial.

Treatment of symptom-free individuals

Symptom-free carriers of *C difficile* are at a relatively low risk of developing CDAD,[37,45] and treatment is not recommended. However, symptom-free, colonised patients may be a source for spread in hospitals,[38] and there have been attempts to interrupt epidemics of CDAD by treating such individuals.[123,124] Treatment with oral vancomycin successfully suppresses the organism but may be followed by extended carriage;[125] metronidazole is ineffective. For these reasons, and because symptom-free people are, in general, less likely to be sources of infection than those who have diarrhoea, we do not regard treating them as a viable option.

Preventive strategies

Implementation of a comprehensive infection control programme that included strict application of universal precautions, periodic educational programmes, phenolic disinfection for environmental cleaning, and strict handwashing was associated with a decrease in the incidence of CDAD from 155 per year to 67 per year in an acute care facility.[126] Use of hypochlorite solution as a disinfectant[127–129] and disposable rectal thermometers[130] also decrease the risk of spread. Because of the central role of antibiotics in predisposing to CDAD, restrictive antibiotic policies[131,132] (eg, restricting clindamycin,[133,134] cephalosporins,[135–137] and gatifloxacin[138]) have been effective in reducing disease.

The apparent role of immunity in controlling CDAD has prompted research into the development of a vaccine. Various vaccines have been tested with some success in animals, including a formalin-inactivated *C difficile* toxoid vaccine,[139] live vaccines with *Vibrio cholerae* and *Salmonella typhimurium* acting as vector strains and expressing an attenuated toxin A,[140,141] and conjugate vaccines combining the non-toxic peptide of toxin A covalently with polysaccharides from pneumococcus, *Shigella flexneri*, and *Escherichia coli*.[142] A parenteral *C difficile* toxoid vaccine has been shown to be highly immunogenic in healthy human volunteers, and a trial is underway to test its efficacy in elderly patients as well as in those with recurrent or relapsing CDAD.[143]

Conclusion

C difficile is an important cause of nosocomial morbidity and mortality, and has been implicated in recent epidemics. Current data support the treatment of CDAD with oral metronidazole in a dose of 1·0–1·5 g daily, with oral vancomycin as a second-line agent, although a search for alternate antimicrobial drugs is underway. Treatment of symptom-free patients is not recommended. Current treatment strategies seem to be increasingly ineffective, especially for patients who have multiple recurrences. Biotherapy and vaccination are currently being explored as treatment options for patients with recurrent disease. Greater attention should be paid to hospital infection control policies and restriction of broad-spectrum antibiotics.

Conflicts of interest

References

1 McFarland LV. Diarrhea acquired in the hospital. *Gastroenterol Clin North Am* 1993; **22:** 563–77.

2 Bartlett JG. *Clostridium difficile*: clinical considerations. *Rev Infect Dis* 1990; **12** (suppl 2): S243–51.

3 Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; **346:** 334–39.

4 Olson MM, Shanholtzer CJ, Lee JT Jr, Gerding DN. Ten years of prospective *Clostridium difficile*-associated disease surveillance and treatment at the Minneapolis VA Medical Center, 1982–1991. *Infect Control Hosp Epidemiol* 1994; **15:** 371–81.

5 Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. *Lancet* 2001; **357:** 189–93.

6 Moshkowitz M, Ben Baruch E, Kline Z, Moshe G, Shimoni Z, Konikoff F. Clinical manifestations and outcome of pseudomembranous colitis in an elderly population in Israel. *Isr Med Assoc J* 2004; **6:** 201–04.

7 Pepin J, Valiquette L, Alary ME, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* 2004; **171:** 466–72.

8 Musher DM, Aslam S, Logan N, et al. Relatively poor outcome after treatment of *Clostridium difficile* colitis with metronidazole. *Clin Infect Dis* 2005; **40:** 1586–90.

9 Pepin J, Alary ME, Valiquette L, et al. Increasing risk of relapse after treatment of *Clostridium difficile* colitis in Quebec, Canada. *Clin Infect Dis* 2005; **40:** 1591–97.

10 Kyne L, Hamel MB, Polavaram R, Kelly CP. Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. *Clin Infect Dis* 2002; **34:** 346–53.

11 Wilcox MH, Cunniffe JG, Trundle C, Redpath C. Financial burden of hospital-acquired *Clostridium difficile* infection. *J Hosp Infect* 1996; **34:** 23–30.

12 Anon. Antibiotics, staphylococcal enteritis and pseudomembranous enterocolitis. *N Engl J Med* 1953; **249:** 37–40.

13 Lundsgaard-Hansen P, Senn A, Roos B, Waller U. Staphylococcic enterocolitis. Report of six cases with two fatalities after intravenous administration of N-(pyrrolidinomethyl) tetracycline. *JAMA* 1960; **173:** 1008–13.

14 Keidan SE, Sutherland IF. Staphylococcal pseudomembranous enterocolitis. *Lancet* 1954; **1:** 1125–26.

15 Williams E. Staphylococcal pseudomembranous enterocolitis complicating treatment with aureomycin. *Lancet* 1954; **1:** 999–1000.

16 Friedell GH, Paige E. Pseudomembranous enterocolitis following antibiotic therapy for pneumonia: report of a case. *Am J Clin Pathol* 1954; **24:** 1159–64.

17 Baden WF. Staphylococcal and subsequent *Candida albicans* enterocolitis complicating novobiocin therapy. *Am J Obstet Gynecol* 1957; **74:** 47–52.

18 Tedesco FJ, Barton RW, Alpers DH. Clindamycin-associated colitis. A prospective study. *Ann Intern Med* 1974; **81:** 429–33.

19 Bartlett JG, Onderdonk AB, Cisneros RL, Kasper DL. Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. *J Infect Dis* 1977; **136:** 701–05.

20 Rifkin GD, Fekety FR, Silva J Jr. Antibiotic-induced colitis implication of a toxin neutralised by *Clostridium sordellii* antitoxin. *Lancet* 1977; **2:** 1103–06.

21 Larson HE, Price AB. Pseudomembranous colitis: presence of clostridial toxin. *Lancet* 1977; **2:** 1312–14.

22 Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978; **298:** 531–34.

23 Bartlett JG, Moon N, Chang TW, Taylor N, Onderdonk AB. Role of *Clostridium difficile* in antibiotic-associated pseudomembranous colitis. *Gastroenterology* 1978; **75:** 778–82.

24 Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr. *Clostridium difficile*-associated diarrhea and colitis. *Infect Control Hosp Epidemiol* 1995; **16:** 459–77.

25 Hospital Infection Control Practices Advisory Committee (HICPAC). Recommendations for preventing the spread of vancomycin resistance. *Infect Control Hosp Epidemiol* 1995; **16:** 105–13.

26 ASHP therapeutic position statement on the preferential use of metronidazole for the treatment of *Clostridium difficile*-associated disease. *Am J Health Syst Pharm* 1998; **55:** 1407–11.

27 Fekety R. Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis. American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 1997; **92:** 739–50.

28 Layton BA McDonald LC, Gerding DN, Liedtke LA, Strausbaugh LJ. Changing patterns of *Clostridium difficile* disease: a report from infectious disease physicians. 42nd Annual Meeting of the Infectious Disease Society of America, Boston, Sept 30–Oct 3, 2004. Abstract 563.

29 Bricker E, Garg R, Nelson R, Loza A, Novak T, Hansen J. Antibiotic treatment for *Clostridium difficile*-associated diarrhea in adults. Cochrane Database Syst Rev. 2005;1:CD004610.

30 Hall IC, O'Toole E. Intestinal flora in new-born infants with a description of a new pathogenic anaerobe: *Bacillus difficilis*. *Am J Dis Child* 1935; **48:** 390–402.

31 Triadafilopoulos G, Pothoulakis C, O'Brien MJ, LaMont JT. Differential effects of *Clostridium difficile* toxins A and B on rabbit ileum. *Gastroenterology* 1987; **93:** 273–79.

32 Pothoulakis C, Sullivan R, Melnick DA, et al. *Clostridium difficile* toxin A stimulates intracellular calcium release and chemotactic response in human granulocytes. *J Clin Invest* 1988; **81:** 1741–45.

33 Miller PD, Pothoulakis C, Baeker TR, LaMont JT, Rothstein TL. Macrophage-dependent stimulation of T cell-depleted spleen cells by *Clostridium difficile* toxin A and calcium ionophore. *Cell Immunol* 1990; **126:** 155–63.

34 McEllistrem MC, Carman RJ, Gerding DN, Genheimer CW, Zheng L. A hospital outbreak of *Clostridium difficile* disease associated with isolates carrying binary toxin genes. *Clin Infect Dis* 2005; **40:** 265–72.

35   Viscidi R, Willey S, Bartlett JG. Isolation rates and toxigenic potential of *Clostridium difficile* isolates from various patient populations. *Gastroenterology* 1981; **81:** 5–9.

36   McFarland LV, Mulligan ME, Kwok RY, Stamm WE. Nosocomial acquisition of *Clostridium difficile* infection. *N Engl J Med* 1989; **320:** 204–10.

37   Johnson S, Clabots CR, Linn FV, Olson MM, Peterson LR, Gerding DN. Nosocomial *Clostridium difficile* colonisation and disease. *Lancet* 1990; **336:** 97–100.

38   Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of *Clostridium difficile* by hospitalized patients: evidence for colonized new admissions as a source of infection. *J Infect Dis* 1992; **166:** 561–67.

39   Riley TV, Cooper M, Bell B, Golledge CL. Community-acquired *Clostridium difficile*-associated diarrhea. *Clin Infect Dis* 1995; **20** (suppl 2)**:** S263–65.

40   Hirschhorn LR, Trnka Y, Onderdonk A, Lee ML, Platt R. Epidemiology of community-acquired *Clostridium difficile*-associated diarrhea. *J Infect Dis* 1994; **169:** 127–33.

41   Johnson S, Gerding DN. *Clostridium difficile*-associated diarrhea. *Clin Infect Dis* 1998; **26:** 1027–34.

42   Kyne L, Sougioultzis S, McFarland LV, Kelly CP. Underlying disease severity as a major risk factor for nosocomial *Clostridium difficile* diarrhea. *Infect Control Hosp Epidemiol* 2002; **23:** 653–59.

43   Mulligan ME, Miller SD, McFarland LV, Fung HC, Kwok RY. Elevated levels of serum immunoglobulins in asymptomatic carriers of *Clostridium difficile*. *Clin Infect Dis* 1993; **16** (suppl 4)**:** S239–44.

44   Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med* 2000; **342:** 390–97.

45   Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. *Lancet* 1998; **351:** 633–36.

46   Warny M, Vaerman JP, Avesani V, Delmee M. Human antibody response to *Clostridium difficile* toxin A in relation to clinical course of infection. *Infect Immun* 1994; **62:** 384–89.

47   Teasley DG, Gerding DN, Olson MM, et al. Prospective randomised trial of metronidazole versus vancomycin for *Clostridium-difficile*-associated diarrhoea and colitis. *Lancet* 1983; **2:** 1043–46.

48   Bartlett JG. Treatment of antibiotic-associated pseudomembranous colitis. *Rev Infect Dis* 1984; **6** (suppl 1)**:** S235–41.

49   Nair S, Yadav D, Corpuz M, Pitchumoni CS. *Clostridium difficile* colitis: factors influencing treatment failure and relapse—a prospective evaluation. *Am J Gastroenterol* 1998; **93:** 1873–76.

50   Wallace JF, Smith RH, Petersdorf RG. Oral administration of vancomycin in the treatment of staphylococcal enterocolitis. *N Engl J Med* 1965; **272:** 1014–15.

51   Khan MY, Hall WH. Staphylococcal enterocolitis: treatment with oral vancomycin. *Ann Intern Med* 1966; **65:** 1–8.

52   Tedesco F, Markham R, Gurwith M, Christie D, Bartlett JG. Oral vancomycin for antibiotic-associated pseudomembranous colitis. *Lancet* 1978; **2:** 226–28.

53   Silva J Jr, Batts DH, Fekety R, Plouffe JF, Rifkin GD, Baird I. Treatment of *Clostridium difficile* colitis and diarrhea with vancomycin. *Am J Med* 1981; **71:** 815–22.

54   Fekety R, Silva J, Buggy B, Deery HG. Treatment of antibiotic-associated colitis with vancomycin. *J Antimicrob Chemother* 1984; **14** (suppl D)**:** 97–102.

55   Fekety R, Silva J, Kauffman C, Buggy B, Deery HG. Treatment of antibiotic-associated *Clostridium difficile* colitis with oral vancomycin: comparison of two dosage regimens. *Am J Med* 1989; **86:** 15–19.

56   Young GP, Ward PB, Bayley N, et al. Antibiotic-associated colitis due to *Clostridium difficile*: double-blind comparison of vancomycin with bacitracin. *Gastroenterology* 1985; **89:** 1038–45.

57   Dudley MN, McLaughlin JC, Carrington G, Frick J, Nightingale CH, Quintiliani R. Oral bacitracin vs vancomycin therapy for *Clostridium difficile*-induced diarrhea. A randomized double-blind trial. *Arch Intern Med* 1986; **146:** 1101–04.

58   de Lalla F, Nicolin R, Rinaldi E, et al. Prospective study of oral teicoplanin versus oral vancomycin for therapy of pseudomembranous colitis and *Clostridium difficile*-associated diarrhea. *Antimicrob Agents Chemother* 1992; **36:** 2192–96.

59   Cherry RD, Portnoy D, Jabbari M, Daly DS, Kinnear DG, Goresky CA. Metronidazole: an alternate therapy for antibiotic-associated colitis. *Gastroenterology* 1982; **82:** 849–51.

60   Wenisch C, Parschalk B, Hasenhundl M, Hirschl AM, Graninger W. Comparison of vancomycin, teicoplanin, metronidazole, and fusidic acid for the treatment of *Clostridium difficile*-associated diarrhea. *Clin Infect Dis* 1996; **22:** 813–18.

61   Fernandez A, Anand G, Friedenberg F. Factors associated with failure of metronidazole in *Clostridium difficile*-associated disease. *J Clin Gastroenterol* 2004; **38:** 414–18.

62   Bartlett JG, Tedesco FJ, Shull S, Lowe B, Chang T. Symptomatic relapse after oral vancomycin therapy of antibiotic-associated pseudomembranous colitis. *Gastroenterology* 1980; **78:** 431–34.

63   de Lalla F, Privitera G, Rinaldi E, Ortisi G, Santoro D, Rizzardini G. Treatment of *Clostridium difficile*-associated disease with teicoplanin. *Antimicrob Agents Chemother* 1989; **33:** 1125–27.

64   McFarland LV, Surawicz CM, Greenberg RN, et al. A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA* 1994; **271:** 1913–18.

65   Noren T, Akerlund T, Back E, et al. Molecular epidemiology of hospital-associated and community-acquired *Clostridium difficile* infection in a Swedish county. *J Clin Microbiol* 2004; **42:** 3635–43.

66   Dzink J, Bartlett JG. In vitro susceptibility of *Clostridium difficile* isolates from patients with antibiotic-associated diarrhea or colitis. *Antimicrob Agents Chemother* 1980; **17:** 695–98.

67   Biavasco F, Manso E, Varaldo PE. In vitro activities of ramoplanin and four glycopeptide antibiotics against clinical isolates of *Clostridium difficile*. *Antimicrob Agents Chemother* 1991; **35:** 195–97.

68   Wong SS, Woo PC, Luk WK, Yuen KY. Susceptibility testing of *Clostridium difficile* against metronidazole and vancomycin by disk diffusion and E test. *Diagn Microbiol Infect Dis* 1999; **34:** 1–6.

69   Cheng SH, Chu FY, Lo SH, Lu JJ. Antimicrobial susceptibility of *Clostridium difficile* by E test. *J Microbiol Immunol Infect* 1999; **32:** 116–20.

70   Barbut F, Decre D, Burghoffer B, et al. Antimicrobial susceptibilities and serogroups of clinical strains of *Clostridium difficile* isolated in France in 1991 and 1997. *Antimicrob Agents Chemother* 1999; **43:** 2607–11.

71   Marchese A, Salerno A, Pesce A, Debbia EA, Schito GC. In vitro activity of rifaximin, metronidazole and vancomycin against *Clostridium difficile* and the rate of selection of spontaneously resistant mutants against representative anaerobic and aerobic bacteria, including ammonia-producing species. *Chemotherapy* 2000; **46:** 253–66.

72   Jamal WY, Mokaddas EM, Verghese TL, Rotimi VO. In vitro activity of 15 antimicrobial agents against clinical isolates of *Clostridium difficile* in Kuwait. *Int J Antimicrob Agents* 2002; **20:** 270–74.

73   Pelaez T, Alcala L, Alonso R, Rodriguez-Creixems M, Garcia-Lechuz JM, Bouza E. Reassessment of *Clostridium difficile* susceptibility to metronidazole and vancomycin. *Antimicrob Agents Chemother* 2002; **46:** 1647–50.

74   Subcommittee on Prevention and Control of Antimicrobial-resistant Microorganisms in Hospitals. Preventing the spread of vancomycin resistance: report from the Hospital Infection Control Practices Advisory Committee. *Fed Regist* 1994; **59:** 25758–63.

75   Gerding DN. Is there a relationship between vancomycin-resistant enterococcal infection and *Clostridium difficile* infection? *Clin Infect Dis* 1997; **25** (suppl 2)**:** S206–10.

76   Olsson-Liljequist B, Nord CE. In vitro susceptibility of anaerobic bacteria to nitroimidazoles. *Scand J Infect Dis Suppl* 1981; **26:** 42–45.

77   Brazier JS, Fawley W, Freeman J, Wilcox MH. Reduced susceptibility of *Clostridium difficile* to metronidazole. *J Antimicrob Chemother* 2001; **48:** 741–42.

78   Sanchez JL, Gerding DN, Olson MM, Johnson S. Metronidazole susceptibility in *Clostridium difficile* isolates recovered from cases of *C. difficile*-associated disease treatment failures and successes. *Anaerobe* 1999; **5:** 205–08.

79   Mattila J, Mannisto PT, Mantyla R, Nykanen S, Lamminsivu U. Comparative pharmacokinetics of metronidazole and tinidazole as influenced by administration route. *Antimicrob Agents Chemother* 1983; **23:** 721–25.

80 Loft S, Dossing M, Poulsen HE, et al. Influence of dose and route of administration on disposition of metronidazole and its major metabolites. *Eur J Clin Pharmacol* 1986; **30:** 467–73.

81 Bolton RP, Culshaw MA. Faecal metronidazole concentrations during oral and intravenous therapy for antibiotic associated colitis due to *Clostridium difficile. Gut* 1986; **27:** 1169–72.

82 Friedenberg F, Fernandez A, Kaul V, Niami P, Levine GM. Intravenous metronidazole for the treatment of *Clostridium difficile* colitis. *Dis Colon Rectum* 2001; **44:** 1176–80.

83 Guzman R, Kirkpatrick J, Forward K, Lim F. Failure of parenteral metronidazole in the treatment of pseudomembranous colitis. *J Infect Dis* 1988; **158:** 1146–47.

84 Chang TW, Gorbach SL, Bartlett JG, Saginur R. Bacitracin treatment of antibiotic-associated colitis and diarrhea caused by *Clostridium difficile* toxin. *Gastroenterology* 1980; **78:** 1584–86.

85 Wullt M, Odenholt I. A double-blind randomized controlled trial of fusidic acid and metronidazole for treatment of an initial episode of *Clostridium difficile*-associated diarrhoea. *J Antimicrob Chemother* 2004; **54:** 211–16.

86 Rossignol JF, Maisonneuve H. Nitazoxanide in the treatment of *Taenia saginata* and *Hymenolepis nana* infections. *Am J Trop Med Hyg* 1984; **33:** 511–12.

87 Rossignol JF, Hidalgo H, Feregrino M, et al. A double-'blind' placebo-controlled study of nitazoxanide in the treatment of cryptosporidial diarrhoea in AIDS patients in Mexico. *Trans R Soc Trop Med Hyg* 1998; **92:** 663–66.

88 Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Giardia intestinalis* and *Entamoeba histolytica* or *E. dispar*: a randomized, double-blind, placebo-controlled study of nitazoxanide. *J Infect Dis* 2001; **184:** 381–84.

89 Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of nitazoxanide. *J Infect Dis* 2001; **184:** 103–06.

90 McVay CS, Rolfe RD. In vitro and in vivo activities of nitazoxanide against *Clostridium difficile. Antimicrob Agents Chemother* 2000; **44:** 2254–58.

91 Dubreuil L, Houcke I, Mouton Y, Rossignol JF. In vitro evaluation of activities of nitazoxanide and tizoxanide against anaerobes and aerobic organisms. *Antimicrob Agents Chemother* 1996; **40:** 2266–70.

92 Broekhuysen J, Stockis A, Lins RL, De Graeve J, Rossignol JF. Nitazoxanide: pharmacokinetics and metabolism in man. *Int J Clin Pharmacol Ther* 2000; **38:** 387–94.

93 Trudel JL, Deschenes M, Mayrand S, Barkun AN. Toxic megacolon complicating pseudomembranous enterocolitis. *Dis Colon Rectum* 1995; **38:** 1033–38.

94 Elinav E, Planer D, Gatt ME. Prolonged ileus as a sole manifestation of pseudomembranous enterocolitis. *Int J Colorectal Dis* 2004; **19:** 273–76.

95 Salcedo J, Keates S, Pothoulakis C, et al. Intravenous immunoglobulin therapy for severe *Clostridium difficile* colitis. *Gut* 1997; **41:** 366–70.

96 Wilcox MH. Descriptive study of intravenous immunoglobulin for the treatment of recurrent *Clostridium difficile* diarrhoea. *J Antimicrob Chemother* 2004; **53:** 882–84.

97 Kelly CP, Pothoulakis C, Vavva F, et al. Anti-*Clostridium difficile* bovine immunoglobulin concentrate inhibits cytotoxicity and enterotoxicity of *C. difficile* toxins. *Antimicrob Agents Chemother* 1996; **40:** 373–79.

98 Lyerly DM, Bostwick EF, Binion SB, Wilkins TD. Passive immunization of hamsters against disease caused by *Clostridium difficile* by use of bovine immunoglobulin G concentrate. *Infect Immun* 1991; **59:** 2215–18.

99 Babcock GJ, Coccia JA, Esshaki DJ, et al. Human monoclonal antibody against toxin a protects hamsters from *Clostridium difficile* disease. 42nd Annual Meeting of the Infectious Disease Society of America, Boston, Sept 30–Oct 3, 2004. Abstract 567.

100 Bartlett JG, Chang TW, Onderdonk AB. Comparison of five regimens for treatment of experimental clindamycin-associated colitis. *J Infect Dis* 1978; **138:** 81–86.

101 Mogg GA, Arabi Y, Youngs D, et al. Therapeutic trials of antibiotic associated colitis. *Scand J Infect Dis Suppl* 1980; (suppl 22): 41–45.

102 Mogg GA, George RH, Youngs D, et al. Randomized controlled trial of colestipol in antibiotic-associated colitis. *Br J Surg* 1982; **69:** 137–39.

103 Taylor NS, Bartlett JG. Binding of *Clostridium difficile* cytotoxin and vancomycin by anion-exchange resins. *J Infect Dis* 1980; **141:** 92–97.

104 Cavagnaro C, Berezin S, Medow MS. Corticosteroid treatment of severe, non-responsive *Clostridium difficile* induced colitis. *Arch Dis Child* 2003; **88:** 342–44.

105 Cozart JC, Kalangi SS, Clench MH, et al. *Clostridium difficile* diarrhea in patients with AIDS versus non-AIDS controls. Methods of treatment and clinical response to treatment. *J Clin Gastroenterol* 1993; **16:** 192–94.

106 Lu SS, Schwartz JM, Simon DM, Brandt LJ. *Clostridium difficile*-associated diarrhea in patients with HIV positivity and AIDS: a prospective controlled study. *Am J Gastroenterol* 1994; **89:** 1226–29.

107 Fekety R, McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Mulligan ME. Recurrent *Clostridium difficile* diarrhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blinded trial. *Clin Infect Dis* 1997; **24:** 324–33.

108 Johnson S, Adelmann A, Clabots CR, Peterson LR, Gerding DN. Recurrences of *Clostridium difficile* diarrhea not caused by the original infecting organism. *J Infect Dis* 1989; **159:** 340–43.

109 O'Neill GL, Beaman MH, Riley TV. Relapse versus reinfection with *Clostridium difficile. Epidemiol Infect* 1991; **107:** 627–35.

110 Barbut F, Richard A, Hamadi K, Chomette V, Burghoffer B, Petit JC. Epidemiology of recurrences or reinfections of *Clostridium difficile*-associated diarrhea. *J Clin Microbiol* 2000; **38:** 2386–88.

111 Tang-Feldman Y, Mayo S, Silva J Jr, Cohen SH. Molecular analysis of *Clostridium difficile* strains isolated from 18 cases of recurrent *Clostridium difficile*-associated diarrhea. *J Clin Microbiol* 2003; **41:** 3413–14.

112 McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent *Clostridium difficile* disease. *Am J Gastroenterol* 2002; **97:** 1769–75.

113 Buggy BP, Fekety R, Silva J Jr. Therapy of relapsing *Clostridium difficile*-associated diarrhea and colitis with the combination of vancomycin and rifampin. *J Clin Gastroenterol* 1987; **9:** 155–59.

114 Persky SE, Brandt LJ. Treatment of recurrent *Clostridium difficile*-associated diarrhea by administration of donated stool directly through a colonoscope. *Am J Gastroenterol* 2000; **95:** 3283–85.

115 Aas J, Gessert CE, Bakken JS. Recurrent *Clostridium difficile* colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. *Clin Infect Dis* 2003; **36:** 580–85.

116 Seal D, Borriello SP, Barclay F, Welch A, Piper M, Bonnycastle M. Treatment of relapsing *Clostridium difficile* diarrhoea by administration of a non-toxigenic strain. *Eur J Clin Microbiol* 1987; **6:** 51–53.

117 Pochapin M. The effect of probiotics on *Clostridium difficile* diarrhea. *Am J Gastroenterol* 2000; **95** (suppl 1)**:** S11–13.

118 Wullt M, Hagslatt ML, Odenholt I. *Lactobacillus plantarum* 299v for the treatment of recurrent *Clostridium difficile*-associated diarrhoea: a double-blind, placebo-controlled trial. *Scand J Infect Dis* 2003; **35:** 365–67.

119 Plummer S, Weaver MA, Harris JC, Dee P, Hunter J. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. *Int Microbiol* 2004; **7:** 59–62.

120 D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *BMJ* 2002; **324:** 1361.

121 Beales IL. Intravenous immunoglobulin for recurrent *Clostridium difficile* diarrhoea. *Gut* 2002; **51:** 456.

122 Leung DY, Kelly CP, Boguniewicz M, Pothoulakis C, LaMont JT, Flores A. Treatment with intravenously administered gamma globulin of chronic relapsing colitis induced by *Clostridium difficile* toxin. *J Pediatr* 1991; **118:** 633–37.

123 Delmee M, Vandercam B, Avesani V, Michaux JL. Epidemiology and prevention of *Clostridium difficile* infections in a leukemia unit. *Eur J Clin Microbiol* 1987; **6:** 623–27.

124 Kerr RB, McLaughlin DI, Sonnenberg LW. Control of *Clostridium difficile* colitis outbreak by treating asymptomatic carriers with metronidazole. *Am J Infect Control* 1990; **18:** 332–35.

125 Johnson S, Homann SR, Bettin KM, et al. Treatment of asymptomatic *Clostridium difficile* carriers (fecal excretors) with vancomycin or metronidazole. A randomized, placebo-controlled trial. *Ann Intern Med* 1992; **117:** 297–302.

126 Zafar AB, Gaydos LA, Furlong WB, Nguyen MH, Mennonna PA. Effectiveness of infection control program in controlling nosocomial *Clostridium difficile. Am J Infect Control* 1998; **26:** 588–93.

127 Kaatz GW, Gitlin SD, Schaberg DR, et al. Acquisition of *Clostridium difficile* from the hospital environment. *Am J Epidemiol* 1988; **127:** 1289–94.

128 Mayfield JL, Leet T, Miller J, Mundy LM. Environmental control to reduce transmission of *Clostridium difficile. Clin Infect Dis* 2000; **31:** 995–1000.

129 Wilcox MH, Fawley WN, Wigglesworth N, Parnell P, Verity P, Freeman J. Comparison of the effect of detergent versus hypochlorite cleaning on environmental contamination and incidence of *Clostridium difficile* infection. *J Hosp Infect* 2003; **54:** 109–14.

130 Brooks SE, Veal RO, Kramer M, Dore L, Schupf N, Adachi M. Reduction in the incidence of *Clostridium difficile*-associated diarrhea in an acute care hospital and a skilled nursing facility following replacement of electronic thermometers with single-use disposables. *Infect Control Hosp Epidemiol* 1992; **13:** 98–103.

131 McNulty C, Logan M, Donald IP, et al. Successful control of *Clostridium difficile* infection in an elderly care unit through use of a restrictive antibiotic policy. *J Antimicrob Chemother* 1997; **40:** 707–11.

132 Carling P, Fung T, Killion A, Terrin N, Barza M. Favorable impact of a multidisciplinary antibiotic management program conducted during 7 years. *Infect Control Hosp Epidemiol* 2003; **24:** 699–706.

133 Pear SM, Williamson TH, Bettin KM, Gerding DN, Galgiani JN. Decrease in nosocomial *Clostridium difficile*-associated diarrhea by restricting clindamycin use. *Ann Intern Med* 1994; **120:** 272–77.

134 Climo MW, Israel DS, Wong ES, Williams D, Coudron P, Markowitz SM. Hospital-wide restriction of clindamycin: effect on the incidence of *Clostridium difficile*-associated diarrhea and cost. *Ann Intern Med* 1998; **128:** 989–95.

135 O'Connor KA, Kingston M, O'Donovan M, Cryan B, Twomey C, O'Mahony D. Antibiotic prescribing policy and *Clostridium difficile* diarrhoea. *QJM* 2004; **97:** 423–29.

136 Wilcox MH, Freeman J, Fawley W, et al. Long-term surveillance of cefotaxime and piperacillin-tazobactam prescribing and incidence of *Clostridium difficile* diarrhoea. *J Antimicrob Chemother* 2004; **54:** 168–72.

137 Khan R, Cheesbrough J. Impact of changes in antibiotic policy on *Clostridium difficile*-associated diarrhoea (CDAD) over a five-year period in a district general hospital. *J Hosp Infect* 2003; **54:** 104–08.

138 Gaynes R, Rimland D, Killum E, et al. Outbreak of *Clostridium difficile* infection in a long-term care facility: association with gatifloxacin use. *Clin Infect Dis* 2004; **38:** 640–45.

139 Torres JF, Lyerly DM, Hill JE, Monath TP. Evaluation of formalin-inactivated *Clostridium difficile* vaccines administered by parenteral and mucosal routes of immunization in hamsters. *Infect Immun* 1995; **63:** 4619–27.

140 Ryan ET, Butterton JR, Smith RN, Carroll PA, Crean TI, Calderwood SB. Protective immunity against *Clostridium difficile* toxin A induced by oral immunization with a live, attenuated *Vibrio cholerae* vector strain. *Infect Immun* 1997; **65:** 2941–49.

141 Ward SJ, Douce G, Figueiredo D, Dougan G, Wren BW. Immunogenicity of a *Salmonella typhimurium aroA aroD* vaccine expressing a nontoxic domain of *Clostridium difficile* toxin A. *Infect Immun* 1999; **67:** 2145–52.

142 Pavliakova D, Moncrief JS, Lyerly DM, et al. *Clostridium difficile* recombinant toxin A repeating units as a carrier protein for conjugate vaccines: studies of pneumococcal type 14, *Escherichia coli* K1, and *Shigella flexneri* type 2a polysaccharides in mice. *Infect Immun* 2000; **68:** 2161–66.

143 Aboudola S, Kotloff KL, Kyne L, et al. *Clostridium difficile* vaccine and serum immunoglobulin G antibody response to toxin A. *Infect Immun* 2003; **71:** 1608–10.