Probiotics: An Alternative to Antibiotics for the Treatment and Prophylaxis of Clostridium difficile-Associated Disease

by

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List of Abbreviations

AAC: antibiotic-associated colitis
AAD: antibiotic-associated diarrhea
AAPMC: antibiotic-associated pseudomembranous colitis
CDAD: Clostridium difficile-associated disease
CF: continuous-flow
CI: confidence interval
EHC: enterohepatic circulation
ICU: intensive care unit
LTCF: long-term care facility
mRR: multivariate-adjusted relative risk
OR: odds ratio
PMC: pseudomembranous colitis
PSE: portal-systemic encephalopathy
REA: restriction endonuclease assay
RR: relative risk
Statement of Purpose

A probiotic is "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance." This document purports to review probiotic approaches to preventing and treating Clostridium difficile-associated disease. The salient clinical and epidemiological aspects of C. difficile-associated disease will be presented, with emphasis placed on those features most relevant to probiotic intervention. Because probiotics exert their effects by altering the composition and metabolic actions of the intestinal microflora, the role of the intestinal microflora in health and disease is briefly considered. Finally, the clinical experience with four different probiotic preparations used to prophylax or treat C. difficile-associated disease is reviewed. The document concludes with a brief recapitulation of the deficiencies in the literature that hamper the formulation of effective prophylactic strategies against C. difficile.
Introduction

Clostridium difficile is an anaerobic, spore-forming, Gram-positive rod. Pathogenic strains elaborate an enterotoxin and a cytotoxin. While C. difficile is widely distributed in nature, only 2–5% of the general population carries it. The situation changes dramatically in the hospital, where the carriage rate among patients can surpass 20%.

Clinical

The majority of these carriers are asymptomatic. Asymptomatic carriage, however, is just one possible outcome of C. difficile infection. This pathogen is the foremost single cause of hospital-acquired diarrhea, accounting for 20% of all cases. The clinical spectrum of C. difficile-associated disease (CDAD) varies from mild diarrhea that resolves readily to protracted diarrhea occurring 20–30 times a day over the course of months. In addition, C. difficile can cause colitis, an inflammation of the large intestine. Pseudomembranous colitis (PMC) is a deadly form of colitis associated almost exclusively with C. difficile.

Clinical complications of CDAD include electrolyte imbalance, dehydration, protein-losing enteropathy, toxic megacolon, and colonic perforation. These complications contribute to the 25–35% case fatality rate associated with CDAD. Antibiotics are the standard therapy for serious or prolonged CDAD. Up to 55% of patients
receiving such therapy will relapse after discontinuing treatment. Multiple relapses are reported to occur in 11% of patients.

**Epidemiology**

Not only is CDAD potentially fatal, but it is common as well. Published incidences range from 0.01 to 10% of hospital admissions. This wide range, spanning two orders of magnitude, is largely due to the epidemic outbreaks of CDAD that occasionally punctuate the underlying endemic disease rates plaguing most hospitals. And even within hospitals, the infection rates are not uniform across the various wards. The rates within a given ward will vary over time.

This behavior attests to the fact that *C. difficile* is predominantly a nosocomial pathogen. Transmission routes include direct and indirect contact spread exemplified by person-to-person and enteric instrumentation contamination, respectively. Common-vehicle spread has also been documented. Hospital infection control measures targeting these modes of transmission have been associated with both significantly reduced rates of endemic CDAD and with the resolution of hospital outbreaks.

Antibiotics are the best established risk factor for *C. difficile* infection and disease, which is extremely rare in patients who have not had a recent course of antibiotics. Although broad-spectrum antibiotics such as clindamycin, cephalosporins, and ampicillin are clearly associated with the greatest risk, almost all antibiotics have been implicated.

Despite the intimate association between CDAD and antibiotic usage, antibiotic-associated diarrhea (AAD) and colitis (AAC) are not
synonymous with CDAD. Diarrhea of all etiologies develops in up to 25% of hospitalized patients receiving antibiotics.

Age is another important risk factor associated with *C. difficile* infection and disease. Infants have carriage rates of toxigenic *C. difficile* approaching 50%, yet most remain asymptomatic, even when they have high levels of *C. difficile* toxins in their stools. It is believed that the intestinal cells of infants lack the receptors for the toxins. The incidence of CDAD increases with age, independent of the increased frequency and duration of hospital visits.

Additional host factors positively associated with *C. difficile* infection and disease include length of hospital stay, severity of underlying disease, in-hospital procedures, antacids, stool softeners, gastrointestinal manipulations, cancer chemotherapy, and renal disorders. Recent infection with *C. difficile* may possibly represent a risk of CDAD. Gender and immunocompromised state are not associated with CDAD, and the risk associated with a history of prior *C. difficile* infection remains controversial.

No dominantly epidemic strains of *C. difficile* have been identified. Yet the various strains of *C. difficile* clearly differ with respect to toxin production *in vitro* and *in vivo*, though again, no particularly virulent strains have been isolated. Strains shed by asymptomatic carriers are infectious and can cause severe disease in susceptible patients.

**Intestinal Microflora**

The most widely accepted explanation for how antibiotics predispose to *C. difficile* infection and disease concerns their actions
on the normal intestinal microflora. Antibiotics disrupt this complex bacterial community, which plays an important role in the health and disease of their human host.

Particularly relevant to the pathogenesis of CDAD, the microflora endows the host with colonization resistance against exogenous pathogens such as *C. difficile*. They accomplish this protective effect by competing with the invader for nutrients and colonic adhesions sites, as well as by secreting metabolites toxic to exogenous bacteria.

Various factors besides antibiotics affect the composition and metabolic activity of the microflora: diet, stress, age, and physicochemical changes such as pH, motility, and oxidation-reduction potential. The intestinal microflora, in turn, plays a significant role in intestinal development, nutrition, carcinogenesis, and drug pharmacokinetics.

**Probiotics**

Probiotics are defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." Four probiotics have been advanced in the literature as effective agents against CDAD. *Saccharomyces boulardii* is a yeast believed to interfere with the action of *C. difficile*'s toxins. Lactobacilli preparations of various kinds may act by interacting with the intestinal flora, thereby bolstering colonization resistance. Fecal enemas restore colonization resistance by replacing the disrupted bacterial flora. Nontoxigenic strains of *C. difficile* compete
with pathogenic strains for the same nutrients and adhesion sites, thereby preventing them from infecting the host.
I--Clinical Aspects of *Clostridium difficile*-Associated Disease

**History**

*Clostridium difficile* entered the world of microbiology, without much fanfare, in 1935 (Hall and O'Toole 1935). The bacterium was believed to be part of the normal intestinal flora of newborn infants, and was christened "difficile" because of its fastidious growth requirements. Subsequently it was found to elaborate a "neurotoxin" which, when injected into rodents, caused convulsions, respiratory arrest, and death (Snyder 1937). Nevertheless, because it was not considered to be a human pathogen, the bacterium received little attention until the 1970s (Lyerly et al. 1988).

Pseudomembranous colitis (PMC), on which the notoriety of *C. difficile* would come to rest, received somewhat more attention. The disease was first reported in 1893, as a postoperative complication characterized by hemorrhagic diarrhea and an intestinal membrane reminiscent of diphtheria (Finney 1893). During the preantibiotic era intestinal surgery was the most common association in the few reported cases of PMC (Penner and Bernheim 1939). After World War II, proportionately more presentations of PMC became associated with antibiotic therapy as the use this treatment burgeoned. While this association with antibiotic use did not escape notice, it was dramatically brought to attention in 1974, with the publication of a prospective study of 200 patients receiving clindamycin, 11% of whom developed PMC (Tedesco et al. 1974).

Before this seminal publication, *Staphylococcus aureus* had been the suspected etiological agent of PMC. This perception changed when Tedesco et
al. (1974) were unable to culture *S. aureus* from biopsies of the pseudomembranes, smears of the intestinal mucosa, or stools of the patients with PMC (Bartlett 1988). The stools of patients with PMC were shown to have high levels of cytotoxic activity (Larson et al. 1977). Eventually, several laboratories reported that antisera raised against crude toxin preparations from various species of clostridia could neutralize this activity. Further studies revealed that only antiserum to *Clostridium sordellii* could neutralize the cytotoxicity of the PMC stools. The problem was that this species was almost never isolated from patients with PMC. Ultimately, *C. difficile* was identified as the source of the cytotoxin and, by extension, the cause of PMC (Bartlett et al. 1978, George et al. 1978).

**Spectrum of Disease**

PMC is only the most extreme outcome associated with antibiotic therapy. Far more common but less life-threatening are antibiotic-associated diarrhea (AAD) and antibiotic-associated colitis (AAC). However, the most common outcome is asymptomatic carriage.

*Clostridium difficile* has been implicated as an etiological agent in all these antibiotic-associated intestinal pathologies, although the specificity of the association decreases with the decreasing severity of the condition. Thus, while evidence of *C. difficile* complicity is found in nearly 100% of cases of PMC, this figure drops to 20% for AAD (Bartlett 1992).

While the preponderance of *C. difficile*-associated disease (CDAD) is attributed to antibiotic exposure, the literature contains numerous instances of disease in patients taking other drugs, including cytotoxic agents (Andrejak et al. 1991, Barc et al. 1992) and neuroleptics (Barc et al. 1992). In addition, *C. difficile* has been weakly associated with a variety of diseases, including necrotizing
enterocolitis (Han et al. 1983) and Hirschsprung's (Hardy et al. 1993) in infants and children and inflammatory bowel disease (Trnka and LaMont 1981) in adults.

The symptoms of CDAD often begin after 5 to 10 days of antibiotic therapy, although cases have been reported as early as the first day and as late as 10 weeks after the therapy is discontinued (Knoop et al. 1993). Diarrhea is the hallmark of CDAD. The severity of the diarrhea can vary from a brief, self-limited bout of loose-stools to diarrhea reminiscent of cholera by having over 20 watery stools a day, though unlike cholera, the diarrhea of CDAD can last for months (Bartlett 1992). *Clostridium difficile* is one of the few enteric bacterial pathogens that frequently causes chronic disease (Bartlett 1990).

Colitis is associated with crampy abdominal pain and devastating diarrhea of the type mentioned above. Occult colonic bleeding may be detected, but frankly bloody stools are uncommon (Kelly et al. 1994). Patients commonly have systemic manifestations such as fever, nausea, loss of appetite, and malaise (Bartlett 1992). Circulating levels of polymorphonuclear cells are often elevated, and these leukocytes are commonly found in the stools as well (Kelly et al. 1994).

The diagnosis of PMC cannot be made clinically: visualization with sigmoidoscopy or colonoscopy is necessary to make the diagnosis (Tedesco et al. 1974). These techniques show yellow adherent plaques varying in diameter from 2 to 10 mm which, in severe cases, may coalesce (Kelly et al. 1994). While the rectum and sigmoid colon are the most common sites for the plaques, they may be confined to the proximal colon in 10% of cases (Kelly et al. 1994). Clinically, PMC presents like colitis without pseudomembranes, only more severe.

The complications of CDAD can be life-threatening. Dehydration and electrolyte imbalance are complications that can arise from any protracted bout of diarrhea, and, depending upon the underlying condition of the patient, can
have serious consequences. More specific in nature is toxic megacolon, wherein a loss of colonic muscular tone leads to dilation of the colon and thinning of the wall. The condition does not always resolve with medical intervention, in which case surgery is required. Tragically, because the loss of muscular tone can lead to a decrease in diarrhea, the diagnosis of toxic megacolon can be missed, with fatal results: failure to promptly reverse the megacolon can result in perforation of the bowel.

Protein-losing enteropathy has been reported in patients infected with C. difficile; while the degree of protein loss increased with the severity of the CDAD, 50% of the patients tested had detectable protein loss in their feces despite having well-formed stools (Rybolt et al. 1989). Protein-losing enteropathy is found in several gastrointestinal diseases causing mucosal destruction and inflammation, and may account for the hypoalbuminemia and anasarca developing in the late stages of PMC. Polyarthritis, which occurs in other forms of colitis, has been rarely attributed to C. difficile colitis as well (Rollins and Moeller 1975, Fairweather et al. 1980).

**Enigmatic AAD**

Although C. difficile is the single greatest identified cause of AAD, it is determined to be the etiological agent of only 20% of cases of AAD. In the vast majority of the 80% of non-C. difficile AAD, no putative agent or recognized pathophysiological mechanism is established, justifying the term "enigmatic diarrhea" (Bartlett 1992). Thus, while Salmonella spp., Clostridium perfringens, Candida albicans, and Staphylococcus aureus also can be causes of AAD, they are very rarely implicated (Bartlett 1992).

Diarrhea associated with C. difficile differs from enigmatic diarrhea in several important ways (Table I-1)(Bartlett 1992). Clinically, enigmatic diarrhea
is rarely accompanied by cramps, fever, or elevated white blood cell counts. Symptoms of enigmatic diarrhea are a function of the antimicrobial dose, and therefore they resolve after the drug is discontinued. Epidemiologically, only C. difficile is responsible for endemic and epidemic outbreaks of diarrhea in hospitals and nursing homes.

For completeness, it should be mentioned that a right-sided hemorrhagic colitis, associated with ampicillin yet endoscopically distinct from PMC with rectal sparing, has been identified in at least 10 patients (Sakurai et al. 1979, Gould et al. 1982). C. difficile has not been implicated (Gerding et al. 1988).

**Antibiotic Toxicity Does Not Exonorate C. difficile**

A *prima facie* explanation for the prominence of antibiotic use in the development of CDAD would posit that antibiotics are directly toxic to the intestinal cells. In such a scenario, C. difficile overgrowth following disruption of the intestinal barriers to colonization is then a finding incidental to the pathogenesis of antibiotic-associated disease, much as *Haemophilus influenzae* turned out to be incidental to the pathogenesis of the 1918 influenza epidemic, from which the bacterium erroneously got its name.

Apparently consistent with such an explanation are the findings that drugs with good activity against C. difficile in vitro may actually cause CDAD (Bartlett 1992). In addition, antibiotics with the same antimicrobial spectrum differ in their propensity to cause CDAD (Table I-2). Furthermore, most of the C. difficile strains isolated from the stools of patients with AAD or AAC were in fact relatively sensitive to the implicated antibiotics (Table I-3) (Dzink and Bartlett 1980). Further support for this hypothesis may be derived from reports that neomycin (Faloon et al. 1966, Robinson et al. 1966, Broitman et al. 1967), tetracycline (Yeh and Shils 1966, Ling and Morin 1971), and penicillin
(Hindmarsh et al. 1967) cause malabsorption by interfering with digestion and absorption of fats or transport of digested carbohydrates and amino acids. Malabsorption is a well established cause of diarrhea. More recently, clindamycin, the antibiotic still most frequently associated with CDAD, was demonstrated to alter water and electrolyte transport in rat intestines, inducing net secretion from the ileum in a dose-dependent manner (Giannella et al. 1981).

Taken as a whole, such arguments seem rather damning for the role of C. difficile in antibiotic-associated disease. However, these arguments are readily refuted.

Starting from the bottom, the disruption of water and electrolyte transport was quickly elicited upon exposure to clindamycin, and likewise, quickly resolved upon its discontinuation (Giannella et al. 1981). This temporal profile is in marked contrast to the kinetics of purported CDAD, which frequently shows a delayed onset of several days, with symptoms sometimes not appearing until discontinuation of the drug (Tedesco et al. 1974, Knoop et al. 1993). Moreover, CDAD is often described as being antibiotic dose-independent (Bartlett 1992), whereas diarrhea associated with clindamycin in human beings has not been found to be dose-dependent (Swartzberg et al. 1977). Presumably, similar arguments would apply to the other transport defects. Thus, while some antibiotics may indeed cause diarrhea by a direct action on enterocyte function, the clinical manifestations of this activity are rather distinct from those of CDAD. It is possible, however, that this mechanism contributes to the etiology of enigmatic diarrhea.

There are a variety of reasons to account for the observation that CDAD occurs even when the isolated strains are susceptible in vitro to the implicated antibiotic. It is a well documented phenomenon that the components of the intestinal microflora will secrete β-lactamases which protect not only the
secreting species, but those in the vicinity as well, against penicillins. The same phenomenon may apply to other antibiotics as well. Moreover, \textit{C. difficile} forms spores when confronted with inhospitable environmental conditions, of which antibiotics are certainly one type. Spores are metabolically inactive and would not be affected by the antibiotics. Thus, assuming that spores are not mechanically cleared from the intestinal tract, antibiotics alone will not rid the body of the infection.

To account for the different frequencies of CDAD induction among antibiotics with similar antimicrobial spectra of action, one must also take into consideration the pharmacokinetics of the drugs, that is, the action of the body on the drug. While two antibiotics may have a similar spectrum of activity, they may have extremely different anatomical sites of action. Important parameters to consider are the degree of absorption from the gastrointestinal tract, the degree of biliary excretion, and the role of metabolic elimination. Thus, some drugs may be fully absorbed from the upper gastrointestinal tract and excreted in the urine, resulting in negligible concentrations in the lower intestines. At the opposite extreme, a drug may not be absorbed at all, resulting in high intestinal concentrations. Between these two possibilities, drugs differ in their degree of bile excretion and secretion by the intestinal mucosa.

\textbf{Pathogenesis}

The pathogenicity of \textit{C. difficile} has been associated with the production of two exotoxins, A and B. Both toxins are large molecular-weight proteins believed to be composed of a single polypeptide. The potency of these lethal toxins are second only to those produced by two other clostridia, \textit{C. botulinum} and \textit{C. tetani} (Gill 1982). Toxin B is a potent cytotoxin: concentrations as low as 50 picograms (50 \times 10^{-12} \text{ grams}) per milliliter will cause cultured cells to undergo
characteristic morphological changes. Although toxin A is best characterized as an enterotoxin, it too possesses cytotoxic activity, albeit significantly less than toxin B. These two actions of toxin A derive from different sites on the molecule (Lyerly et al. 1988). In addition to these two toxins, a motility-altering factor has been described (Justus et al. 1982).

Early observations that the toxins caused rounding of a variety of cultured cell types prompted attention to be focused on the molecule with the more powerful cytotoxic activity, toxin B. Similarly, a major emphasis was placed on elucidating the mechanism of the cytotoxicity in the belief that the basis of the intestinal effects of the toxins lay in their cytotoxicity (Donta 1988).

However, experiments in vivo show that toxin A elicits a greater intestinal response than toxin B. First, the tips of the intestinal villi are damaged, then the brush border membrane is disrupted, and finally the mucosa becomes eroded, resulting in an outpouring of hemorrhagic fluid (Lyerly et al. 1988). Toxin A may also act as a potent activator and chemoattractant for human leukocytes (Knoop et al. 1993).

While the severity of the symptoms elicited in animal models correlates more closely with toxin A than B (Borriello et al. 1985), other studies indicate that toxin B is also important. For example, a vaccine against the two toxins protects hamsters against C. difficile disease; both are necessary for complete protection (Lyerly et al. 1988).

A series of highly provocative studies suggest that the actions of the two toxins are synergistic. Both toxins are lethal at similar doses when injected intravenously, intraperitoneally, or even subcutaneously into rodents or rhesus monkeys (Arnon et al. 1984). Intragastric administration of toxin B alone has no effect on hamsters. However, when the same dose of toxin B is given intragastrically with toxin A at doses too low to produce a discernible response
in hamsters, the combination is deadly. Similar potentiation of toxin B's lethality is obtained if instead of giving toxin A the intestine is damaged by manipulation (Lyerly et al. 1985). Remarkably, the pathologic findings in animals killed by the purified toxins are minimal, regardless of whether the toxins were administered systemically or intragastrically (Lyerly et al. 1985).

Taken together, the above results have been proposed as evidence that toxin A enables toxin B to exit the intestines and act systemically (Lyerly et al. 1988). Beyond their contribution to unravelling the pathogenetic basis of C. difficile disease, these results are potentially important clinically as well. If the toxins do have these devastating extraintestinal effects, infants, who often have fecal levels of cytotoxin comparable to those in adults with PMC, may be placed in great danger by any intestinal manipulation that compromises the integrity of the intestinal mucosa, thereby allowing the cytotoxin to gain access to extraintestinal sites. Indeed, cytotoxin was demonstrated in the serum and ascites fluid of two pediatric patients with fatal PMC (Qualman et al. 1990).

The synergism between toxins A and B nicely complements the observation that their production is coregulated: except under "starved" conditions (Haslam et al. 1986), strains produce either both or none of the toxins (Lyerly et al. 1988). Yet not all strains that carry the genes for the toxins express them in vivo. Moreover, even in vitro, the level of expression varies over six log₁₀ units, depending upon the strain. This range of expression is not seen in other bacteria (Lyerly et al. 1988), and may be important in explaining the wide clinical spectrum of C. difficile disease.

Recovery of the heat-labile toxin of Escherichia coli and the cholera toxin are both increased when the organisms are cultured in the presence of lincomycin, an antibiotic that interferes with protein synthesis (Levner et al. 1977). A similar situation pertains to C. difficile when grown in media
supplemented with clindamycin, an antibiotic related to lincomycin: a 4- to 8-fold increase in toxin expression is observed (Nakamura et al. 1982). Like absolute levels of expression, antibiotic-induced toxin expression is postulated to be strain-specific (Lyerly et al. 1988), which may explain the fact that other authors have failed to discern such an effect (Onderdonk et al. 1981, Lyerly et al. 1988, Barc et al. 1992).

**Treatment**

The first step in treating AAD or AAC is to discontinue antibiotic therapy if possible; if not, an antibiotic that is less prone to cause the disease should be substituted and oral vancomycin therapy begun (Bartlett 1992). Table I-2 lists some common antibiotics and their propensity to cause CDAD. In general, the greater the impact a drug has on the anaerobic flora of the large intestine, the more likely it will be to cause CDAD (Bartlett 1992).

Despite being so intimately associated with antibiotics, CDAD that is associated with serious or persistent symptoms is treated with antibiotics. Rather than culturing the isolate and then determining its susceptibility to various antibiotics, a process which can take weeks, physicians typically treat empirically. Vancomycin and metronidazole are the two most widely used agents, and each has its own advantages and disadvantages. Vancomycin, although very expensive, kills all strains of *C. difficile*. Metronidazole is much cheaper, but resistant strains have been reported (Kelly et al. 1994). Both regimens last 10 to 14 days.

An alternative strategy to targeting the infectious agent is to neutralize the effects of the toxins. Anion-exchange resins that bind both toxins produced by *C. difficile* have been proposed to do just that. Clinical trials, however, rarely gave response rates greater than 50%, and animal tests have shown that
antibiotic regimens are more effective (Finegold and George 1988). Nevertheless, patients who do respond tend not to relapse (Finegold and George 1988). Attempts to combine a resin with vancomycin treatment have been thwarted by the fact that the resins bind the antibiotic as well, significantly decreasing the effective dose (Taylor and Bartlett 1980). Considering this constellation of findings, current recommendations, when they address the use of resins at all, limit the use to patients who are not seriously ill (Finegold and George 1988).

Other therapies aimed at the toxins include the use of C. sordellii antiserum, the same antiserum that is used in the cell culture assay for the cytotoxin. Animal tests were disappointing (Allo et al. 1979, Bartlett 1984b), and the idea has not received additional consideration. Breast milk from postpartum women, which has neutralizing activity against either or both of the toxins, was shown to reduce significantly the accumulation of intestinal fluid in suckling mice challenged with purified toxins (Kim et al. 1984). The protective effect was attributed to the secretory IgA fraction, which suggests that secretory immunity plays a role in recovery from or prevention of CDAD. In fact, stimulation of secretory IgA secretion is one of the methods proposed for the prophylactic measures discussed in section IV.

Whichever treatment modality is finally adopted, the efficacy of the treatment is determined clinically, by the resolution of symptoms. Laboratory-based "test of cure" cultures are of no value (Peterson and Kelly 1993, Kelly et al. 1994), since positive stool cultures or cytotoxin assays can be detected in up to 40% of patients rendered asymptomatic by treatment (Peterson and Kelly 1993), and many of these never have symptomatic relapse (Kelly et al. 1994).
Recurrent Disease

Up to 55% of patients suffer recurrent disease shortly after treatment of CDAD with either metronidazole or vancomycin is discontinued (Young et al. 1985, Johnson and Calia 1989, Bartlett 1990). Sometimes the disease resolves completely with a second round of antibiotic therapy, but 5 to 8% experience five or more relapses (Bartlett 1992). There is currently no consensus on how to treat multiple relapsing CDAD. Proposed modalities include slow tapering doses of vancomycin with or without concurrent use of resins to bind the toxins. All of the probiotic therapies discussed in section IV have also been used (Kelly et al. 1994).

Beyond the morbidity and mortality associated with recurrent CDAD, two aspects of this phenomenon are of particular interest to the focus of this review. Knowledge of the mechanism of recurrent CDAD might prove crucial to establishing appropriate prophylactic measures. Equally important for prophylaxis is identifying a time frame during which patients known to have had CDAD are at increased risk of suffering another bout. Both of these issues will be addressed at greater length in Section III.

Diagnosis of CDAD

Several features of CDAD make diagnosis somewhat problematic. Foremost is the existence of asymptomatic carriage: positive fecal cultures cannot establish the diagnosis. In fact, fecal cytotoxin has also been identified in asymptomatic patients, especially infants. So laboratory tests are by no means sufficient for diagnosis.

Some evidence suggests even that the cytotoxin may not be a necessary finding in CDAD. Endoscopy of symptomatic patients with culture-positive stools revealed pseudomembranes in 11% of the 40 patients with cytotoxin-
negative stools, which, albeit significantly less than the 51% of 109 cytotoxin-positive patients, nonetheless suggests that the detection of cytotoxin is not necessary for a diagnosis of C. difficile-associated PMC (Gerding et al. 1986). Evaluating stool for mucus, occult blood, and fecal leukocytes is also not a sufficiently discriminating test of CDAD (Gerding and Brazier 1993), since these can be found in other types of inflammatory diarrhea.

The above facts demonstrate that laboratory tests alone cannot establish the diagnosis. Furthermore, the tests typically are expensive or time consuming: culturing C. difficile takes 48 hours, while the cytotoxin assay requires tissue culture facilities, which many hospitals still lack. Cheaper and faster albeit less sensitive methods exist for both. These are discussed briefly in Section III. For a more complete discussion, see Peterson and Kelly (1993).

Visualization procedures such as sigmoidoscopy or colonoscopy also cannot establish the diagnosis absolutely, as pseudomembranes are absent in milder or early stages of the disease. At the same time, the diagnosis, even of PMC, cannot be established clinically, since patients with paralytic ileus (Triadafilopilous and Hallstone 1991) or the elderly (Thomas et al. 1990) may develop PMC without associated diarrhea. Symptoms such as fever, abdominal cramps and tenderness, and leukocytosis, and clinical laboratory findings that frequently accompany the disease, may also be absent in PMC (Bartlett 1983). Thus, CDAD must be diagnosed using a combination of laboratory and clinical criteria.
Table I-1. Comparison of cases of antibiotic-associated diarrhea/colitis due to *C. difficile* and enigmatic cases

<table>
<thead>
<tr>
<th>Variable</th>
<th>Due to <em>C. difficile</em></th>
<th>No clear cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs most commonly impicated</td>
<td>Clindamycin, ampicillin, cephalosporins</td>
<td>Clindamycin, tetracycline, ampicillin, some cephalosporins</td>
</tr>
<tr>
<td>Relationship of illness to dose</td>
<td>Not dose-related</td>
<td>Dose-related</td>
</tr>
<tr>
<td>Response to drug withdrawal</td>
<td>Symptoms often persist</td>
<td>Symptoms usually resolve</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>Watery diarrhea and cramps</td>
<td>Watery diarrhea</td>
</tr>
<tr>
<td>Constitutional</td>
<td>Fever and leukocytosis common</td>
<td>Occurrence of symptoms unusual</td>
</tr>
<tr>
<td>History</td>
<td>Usually noncontributory</td>
<td>History of diarrhea with same or other antibiotics often noted</td>
</tr>
<tr>
<td>Complications</td>
<td>Toxic megacolon, ileus, perforation, high fever, leukemoid reaction, dehydration, hypoalbuminemia with anasarca</td>
<td>Rarely seen</td>
</tr>
<tr>
<td>Evidence of colitis</td>
<td>Cramps, white blood cells in feces, colitis or PMC evident on endoscopy with inspection and/or biopsy</td>
<td>Colitis uncommon</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>Epidemic or endemic in hospitals and nursing homes</td>
<td>Sporadic</td>
</tr>
</tbody>
</table>

from Bartlett 1992
<table>
<thead>
<tr>
<th>Common</th>
<th>Less common</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>Penicillins (other than ampicillin)</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Ampicillin/amoxicillin</td>
<td>Erythromycin and other macrolides</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Clindamycin</td>
<td></td>
<td>Quinolones</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulfonamides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rifampin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluorouracil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methotrexate</td>
</tr>
</tbody>
</table>

from Bartlett 1992
Table I-3. Susceptibility of 84 strains of *C. difficile* to 11 antimicrobial agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cumulative % inhibited at conc (mg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>82</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>72</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>60</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>89</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>98</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>92</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>89</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>86</td>
</tr>
</tbody>
</table>

from Dzink and Bartlett 1980
II--Epidemiology

The most salient epidemiologic feature of *C. difficile*-associated disease is that it is predominantly nosocomial in origin: the pathogen is acquired in the hospital. Rates of nosocomial acquisition of *C. difficile* have been reported at 1.1 per 100 person-days (Samore et al. 1994) and 22.7 per 100 patients admitted (MCFarland et al. 1989). While the different denominators make comparisons difficult, it is apparent that these two rates differ substantially when one considers that the mean hospital stay is far shorter than 22 days. Acquisition rates among the elderly range from 0.52/1000 resident-days in a long-term care facility (LTCF) (Simor et al. 1993) to 12.2% on an acute geriatric ward (Rudensky et al. 1993).

The fact that the institutional acquisition rates are so high suggests that the bacterium is not often found in the community. Indeed, some prevalence studies have shown that only 2-5% of the healthy population are carriers of *C. difficile* (Lyerly et al. 1988), though there are indications that rates are much higher in Japan (Nakamura et al. 1981). In the U.S., carrier rates of 5% (MCFarland et al. 1989) or 8% (Samore et al. 1994) have been reported among adult patients entering the hospital at least 60 days after their last admission.

The low prevalence of *C. difficile* carriage in the healthy adult population could represent transient carriage of the general population or prolonged carriage in a subset of the population. A study of healthy neonates found an average colonization period of six months (Knudsen and Tvede 1993). Among children 0 - 14 years old who had gastrointestinal symptoms and culture-positive stools, 87% spontaneously cleared the bacterium within 21 days of first testing positive (Tvede et al. 1990). In adults, 70% of residents in a LTCF for the elderly
carried C. difficile for less than two months (Simor et al. 1993). The data indicate that C. difficile carriage is transient and resolves spontaneously. Ironically, treating asymptomatic carriers with antibiotic regimens similar to those used for symptomatic disease resulted in significantly longer carriage than in subjects treated with placebo (p = 0.05) (Johnson et al. 1992a).

This section will focus on the epidemiological factors associated with C. difficile acquisition, carriage and disease in hospitalized patients. From the standpoint of this review, it is important to identify the risk factors for acquisition in contradistinction to carriage or disease because acquisition represents an additional, and conceptually the ideal, target for prophylactic intervention. Special emphasis will be placed on what has been learned in the past five years, for only in 1989 was the first of several large-scale studies of C. difficile acquisition published. Finally, although C. difficile in infants is epidemiologically very different from that in children and adults, this review will focus on adult populations, since CDAD in infants tends to be more benign (Lyerly et al. 1988).

**Methodological Issues**

A plethora of issues must be considered when evaluating the C. difficile literature. These can be broadly categorized into microbiologic or epidemiologic in nature.

**Microbiological Considerations**

Much of the data on C. difficile requires careful ascertainment of the presence of C. difficile in stool or in the physical environment. Studies on the methods of transmission, for example, require detection of minute amounts of C. difficile or its spores on the hands of health care workers, on the shafts of
thermometers, or on surfaces in hospital rooms. Three techniques have been reported that have been shown to increase the detection of *C. difficile* by up to two orders of magnitude: use of 0.1% sodium taurocholate, alcohol shock, or heat shock (Peterson and Kelly 1993). Because these techniques have not been universally adopted, comparison among studies must be made with caution.

A second issue is the method of detecting the toxins elaborated by *C. difficile*. The most specific method for diagnosing CDAD is the detection of the cytotoxin (Gerding and Brazier 1993), and the gold standard for accomplishing this is cell culture. The assay is very sensitive, detecting picomolar concentrations of the toxin; high specificity is achieved by using antisera prepared against *Clostridium sordelli* toxin, a close homologue of *C. difficile*'s cytotoxin that effectively neutralizes the cytotoxin. But cell culture is expensive and not widely available to hospital laboratories, so this technique is not universally used. A panoply of faster and cheaper commercial alternatives to cell culture is available (Table II-1). Most of them are based on enzyme-linked immunosorbent assays (ELISA) technology. The difference in sensitivity and specificity of these techniques must be borne in mind when making comparisons. The potential for misclassification bias is greater with these alternatives to cell culture.

The most commonly used commercial kit is the Culturette Brand CDT latex agglutination test. It was initially marketed as an alternative to cell culture, but was subsequently found not to be specific for either toxin. Rather, it detected an enzyme that has since been shown to exist in nontoxicogenic strains of *C. difficile* as well (Peterson and Kelly 1993). Nonetheless, because this test detects *C. difficile* regardless of its ability to secrete toxins, the latex agglutination test is now marketed as a fast and inexpensive, albeit less sensitive, alternative to culturing
the organism, which takes 48 hours. The two incarnations of this test compound the confusion in the literature.

Since 1978, when *C. difficile* was identified as the cause of antibiotic-associated pseudomembranous colitis (AAPMC), a number of microbiological techniques have been developed to type isolates of *C. difficile* (Table II-2). Most of these techniques have been used at least once to investigate outbreaks, and have thereby contributed to the understanding of modes of transmission and related issues. However, each system has its short-comings, and no single system has established itself as the definitive typing method. As a result, the literature on the molecular epidemiology of *C. difficile* is in disarray, with very little known about the epidemiology of specific strains. This information is important, insofar as it might identify strains with proclivities to certain subpopulations, modes of transmission, or virulence. Intervention efforts would then be tailored to meet the particular challenges posed by the strains.

Because *C. difficile* is an anaerobe, special precautions must be taken to ensure the viability of the bacterium for culture. Physical factors, such as the size of the stool specimen itself, have an effect on the viability of the organism by affecting the diffusion of oxygen. Likewise, precautions must be taken to preclude the proteolysis of the toxins at room temperature (Gerding and Brazier 1993). Freezing stool samples, however, can lead to a marked decrease in bacterial counts (Edelstein 1988). Variations in the handling of stool samples can therefore have a significant impact on the laboratory's ability to isolate the organism and detect its toxins.

**Epidemiologic Considerations**

One prominent difficulty in the literature concerns case definitions, an essential prerequisite for all epidemiological studies. No consensus exists as to
what constitutes *C. difficile*-associated disease. Some of the literature defines this as culture-positive disease (Thibault et al. 1991), others as cytotoxin-positive diarrhea (Brooks et al. 1992, Brown et al. 1990), and still others require that, in addition to cytotoxin-positive stools, all other possible etiologies be excluded (Johnson et al. 1990a, McFarland et al. 1991, Gerding and Brazier 1993). Even among those who base their definitions on cytotoxin-positive stools, the definition is further eroded by the vagaries of cytotoxin detection methods, with at least one researcher continuing to use the CDT test (Brooks et al. 1992), which, as mentioned above, is not even specific to cytotoxigenic strains, much less the to cytotoxin itself. Beyond the difficulty presented in making comparisons, the use of cytotoxin in the case definition is problematic, insofar as one of the key issues yet to be resolved is the precise role that toxins play in the pathogenesis of CDAD.

Two other terms of crucial importance to the literature are similarly unsettled: "antibiotic-associated" and "diarrhea." With regards to the former, the issue revolves around how remote an exposure is to be considered relevant. There is no standard definition adopted in the literature, and some studies do not even identify their working definition (Fekety et al. 1983, Kim et al. 1981). An additional difficulty is encountered with the definition of diarrhea. Again, some researchers do not even provide one (Brown et al. 1990) while others differ in the number of stools and the time period over which they must be produced.

*Clostridium difficile* has been identified as the cause of hospital outbreaks of CDAD, be it of PMC (Tedesco et al. 1974, Pierce et al. 1982) or AAD (Thibault et al. 1991). Much of the literature contains reports of investigations conducted during such outbreaks but rarely provides a definition of an outbreak. Conversely, one study purporting to investigate the epidemiology *C. difficile*
carriage in an endemic setting reported disease rates that were comparable to those of purported "outbreaks" (Table II-3) (McFarland et al. 1990).

The distinction between epidemic and endemic disease is an important one, as the risk factors and modes of transmission may vary according to the setting. Moreover, since the positive predictive value of a test increases with the prevalence of the tested outcome, it might be hard to distinguish whether a difference between two studies is due to differences in epidemiological factors or to a difference in ability to detect the change.

Some of the literature consists of retrospective studies. Comparisons between cohorts from different years may be vulnerable to bias, since the search for C. difficile etiology may well be initiated at a far lesser degree of severity now than in the past, when C. difficile was a relatively less notorious pathogen.

Because C. difficile causes a wide spectrum of clinical disease that differs both in frequency and specificity for C. difficile, studies may choose to examine any one of several end-points of C. difficile-associated disease. These end-points may differ with respect to risk factors. Cytotoxin, for example, is far more closely associated with an outcome of PMC than it is with AAC or AAD (Table II-4). The relative significance of host factors as opposed to microbial factors might change with the severity of underlying illness.

Because antibiotic use is the major risk factor associated with CDAD and its use may be expected to show seasonal variation, this factor must be taken into account when comparing prevalence rates and when assessing the effects of interventions in controlling hospital outbreaks of AAD.
Host Risk Factors

Antibiotics

Acquisition

An intervention trial randomized 123 people to receive a single dose of prophylactic antibiotics before undergoing elective surgery (Privitera et al. 1991). Volunteers who received cephalosporins were more likely to become colonized with *C. difficile* than those getting mezlocillin ($p = 0.04$). Eighteen of 104 (17.3%) volunteers who received antibiotics became colonized, but none experienced any symptoms. Another experiment in healthy volunteers found that only cephalosporins were significantly associated with acquisition, but in contrast to the results of Privitera et al. (1991) results, 8 of the 15 volunteers who acquired the bacterium were symptomatic (Ambrose et al. 1985). A similar trial in hospitalized patients demonstrated that 5/6 patients receiving cefoxitin, a cephalosporin, acquired *C. difficile* either during or within 1-10 days after therapy (Mulligan et al. 1984); 2/5 developed colitis, while the other three remained asymptomatic.

McFarland and associates (1989) found a bimodal temporal distribution of nosocomial *C. difficile* acquisition, with 40% acquiring the bacterium after two weeks in the hospital. While the authors neglected to report whether antibiotics represented a risk factor for *C. difficile* acquisition in general, they did indicate that patients who acquired *C. difficile* after more than two weeks in the hospital had a greater antibiotic exposure than those who acquired the bacterium within two weeks ($p = 0.001$), especially when the antibiotics were cephalosporins ($p = 0.004$). In addition, they were more likely to have a severe underlying disease ($p < 0.001$) and to be azotemic ($p = 0.04$). Otherwise they were similar for all factors examined.
Samore and colleagues (1994), who did not report any bimodal temporal distribution to *C. difficile* acquisition, found no significant risk associated with either the intensity or the duration of antibiotic therapy. The discrepancy between the McFarland group (1989) and Samore et al. (1994) may be due to the epidemic-like acquisition rates encountered by the former (Table II-3). Alternately, the lack of agreement may stem from Samore and associates’ (1994) lack of a temporal analysis or the use of an enrichment protocol requiring heat-shock at 70°C for 20 minutes, conditions that have been reported to minimize yields of *C. difficile* (Lahn et al. 1993). A third prospective study also failed to demonstrate a significant association between antibiotic use and *C. difficile* acquisition (Rudensky et al. 1993).

Carriage

A variety of studies indicate that antibiotics are a risk factor for carriage (Clabots et al. 1992, Johnson et al. 1990a, Walker et al. 1993, Simor et al. 1993). Two of these studies were prospective cohort studies of 634 (Clabots et al. 1992) and 282 (Johnson et al. 1990a) patients. Johnson and team (1990a), investigating an outbreak among hospitalized adult patients, found that 82% of culture-positive patients had had antibiotics within two weeks of the study, compared to 49% of culture-negative patients (p < 0.001). Clabots and associates’ (1992) rates are somewhat lower, but still achieve a 1% significance. Period prevalence surveys of two LTCF for the elderly found antibiotic exposure to be associated with a risk of 3.31 (95% CI: 1.14, 9.68) (Walker et al. 1993). Cephalosporins and trimethoprim-sulfamethoxazole were associated with a risk of 4.66 and 8.45, respectively (Walker et al. 1993). Simor and colleagues (1993) also studied elderly residents of a LTCF using a combination of prospective cohort and multiple point-prevalence study designs. A multivariate analysis of the 236
specimens collected from 94 residents revealed an adjusted odds ratio of 7.9 for antibiotic exposure within eight weeks of the study, but unlike Walker et al.'s (1993) investigation, no single antibiotic was found to be a significant risk factor.

In contrast, several prospective studies indicated no significant antibiotic use in colonized patients (El-Mohandes et al. 1993, Rudensky et al. 1993, Samore et al. 1994, McFarland et al. 1990). Two of these studies involved over 400 adult patients each (Samore et al. 1994, McFarland et al. 1990), one followed 50 preterm infants (El-Mohandes et al. 1993), and a fourth studied 100 chronic-care geriatric patients (Rudensky et al. 1993).

No straightforward explanation can account for the diametrically opposite results reported by these eight studies. Among those groups not finding a significant association between antibiotic usage and *C. difficile* colonization, one reports that the study was motivated by a steady increase in the number of cases of *C. difficile* diarrhea despite infection control interventions (Samore et al. 1994); another documented acquisition rates comparable to those observed during epidemic outbreaks (McFarland et al. 1990) (Table II-3). These two investigations, therefore, might reflect the risk of antibiotics associated with epidemic conditions. The conclusions of the two other studies that reported no association with antibiotic therapy are problematic as well. El-Mahondes et al. (1993) found that in those infants for whom a third stool sample was available, antibiotics were "markedly" more common in colonized versus non-colonized neonates. Furthermore, his group was investigating a relatively small study population. Similarly, Rudensky and associates' (1993) study suffers from a lack of power: in each of the three wards studied, non-colonized patients outnumber colonized by at least a factor of four, with only three or four colonized patients having received antibiotics.
Antibiotic use may be merely a marker for more severe underlying disease that might predispose patients to colonization by *C. difficile* in a hospital environment. To address this issue the analyses of both McFarland et al. (1989) and Samore and colleagues (1993) used indices to control for the severity of the patients' underlying illness (modified Horn index and the McCabe and Jackson classification, respectively). Among the studies that found a significant association between antibiotics and *C. difficile* carriage, Simor et al. (1993) included in their multivariate analysis a term for the number of underlying diseases, while Walker and associates (1993) controlled for both the amount of nursing time required by the patients and their Horn index. Clabots and company (1992) controlled for the length of hospitalization, which might likewise be a confounder for antibiotics.

Disease

The preponderance of evidence indicates that antibiotics are a risk factor for *C. difficile* diarrhea. Case-control studies demonstrate significant risks associated with the number of antibiotics received (Thibault et al. 1991), the duration of antibiotic treatment regardless of type (Brown et al. 1990), and the type of antibiotic (Thibault et al. 1991, Brown et al. 1990, Hutin et al. 1993). Clindamycin, the quintessential CDAD-causing antibiotic, was associated with odds ratios ranging from 16 to 42 (Thibault et al. 1991, Hutin et al. 1993). Other antibiotics identified by case-control and prospective investigations include neomycin and metronidazole (Thibault et al. 1991); vancomycin, antipseudomonal penicillins, aminoglycosides, and third-generation cephalosporins (Brown et al. 1990); penicillins and cephalosporins (McFarland et al. 1990).
Age and Gender

Acquisition

Neither age nor gender have been found to be significantly associated with acquisition (McFarland et al. 1989, Samore et al. 1994).

Carriage

Using multivariate analysis, one investigation found increasing age to be a significant risk factor for *C. difficile* carriage; gender was not (McFarland et al. 1990). Two studies of elderly LTCF residents (Walker et al. 1993, Simor et al. 1993) and one study of elderly nursing home patients (Thomas et al. 1990) found neither age nor sex to be risk factors. The narrow age range represented in this study population may explain the lack of significance. Yet Samore et al. (1994), who investigated a more diverse population, also found no association between carriage and sex or age.

Disease

Age is frequently cited as a large and significant risk factor for *C. difficile* diarrhea. McFarland et al. (1990) reported a peak mRR of 9.56 (95% CI: 2.17, 42.06) for patients aged 61-75, while Brown and associates (1990) found an adjusted OR of 14.1 (95% CI: 1.4, 141) for age greater than 65. No study of any type found gender to be a significant risk factor.

Exposure to Exogenous Sources

In the past five years additional evidence has supported claims that nosocomially acquired *C. difficile* is transmitted person-to-person. Some investigations report that exposure to a roommate with culture-positive stools is a significant risk factor for nosocomial *C. difficile* acquisition (McFarland et al.)
1989, Clabots et al. 1992); others report an increased yet non-significant risk (Samore et al. 1994). Typing studies have demonstrated acquisition of the same strain as the index case (McFarland et al. 1989, Clabots et al. 1992), even in situations where there was no marked geographical clustering of cases (Clabots et al. 1992). Acquisition rates are greater among patients in double rooms than in single, and the risk of acquiring *C. difficile* after exposure to a culture-positive roommate is significantly greater within the first 48 hours after exposure (McFarland et al. 1989).

An important development in the past five years has been the finding that both symptomatic and asymptomatic carriers of *C. difficile* are a significant source of nosocomially acquired *C. difficile* (McFarland et al. 1989, Clabots et al. 1992). This finding has been demonstrated in settings exhibiting clustering of cases (McFarland et al. 1989) as well as in those not (Clabots et al. 1992).

The distinction between clustered and sporadic cases is an important one to maintain when exploring possible transmission mechanisms. Clustering of *C. difficile* acquisition in individual rooms supports the notion of patient-to-patient transmission, especially when a concordance is found between the *C. difficile* strain infecting the index and secondary case. However, this concordance may be falsely elevated during an outbreak with a single, predominant strain. Finally, to make a convincing case for patient-to-patient transmission, it is necessary to rule out the possibility that the bacterium is being spread by a contaminated health care worker. One investigation reported an absence of geographic clustering but did find several cases of the same strain being acquired in different rooms throughout the ward in the same week (Clabots et al. 1992). Such a pattern suggests spread by a health care worker.

Health care workers have been shown to pick up *C. difficile* on their hands after contact with patients, even after such innocuous-seeming procedures as
taking a temperature, examining patients or feeding them (Mcfarland et al. 1989). The use of gloves by health care workers reduces the level of C. difficile carriage on their hands (Mcfarland et al. 1989) and has been associated with a marked decrease in the incidence of C. difficile diarrhea in an intervention trial (Johnson et al. 1990b). Hand washing with 4% chlorhexidine gluconate, a disinfectant soap, gave far lower rates of recoverable C. difficile than washing with a non-disinfectant (Mcfarland et al. 1989).

Environmental contamination is also a possible source of C. difficile. The literature abounds with reports of C. difficile recovery from surfaces in rooms that housed carriers, and the spores in the hospital environment have been reported to persist for up to six months. Significant environmental contamination was found in rooms previously inhabited by both symptomatic and asymptomatic carriers, and in the majority of cases the immunoblot type matched that of the "epidemiologically-related patient's" isolate (Mcfarland et al. 1989). Environmental contaminants appear to be infectious, since there are reports of patients in non-epidemic settings acquiring the identical strain type as was present in the room (Clabots et al. 1992, Hutin et al. 1993).

Contaminated hospital equipment is another vehicle for transmission. In a study hospital, 21% of electronic rectal thermometers swabbed near the base of the disposable plastic temperature probe sheath yielded positive cultures. Replacing the electric rectal thermometers with disposable ones reduced the incidence of C. difficile diarrhea from 2.71 to 1.76/1000 patient days (p < 0.01) (Brooks et al. 1992).

**Recent Acquisition**

Some authors have suggested that recent acquisition of C. difficile is an important risk factor for subsequent development of disease. Antibiotic-
associated diarrhea usually has its onset within two weeks of beginning antibiotic therapy (Bartlett 1992); since antibiotics are possibly associated with acquisition, this observation is consistent with recent acquisition being a risk factor for disease. In one prospective study, 19 patients acquired toxigenic strains; of these, nine (47%) developed diarrhea in a median of three days (Samore et al. 1994). In contrast, of 24 patients already carrying toxigenic strains at the time of the study, only one developed diarrhea (Samore et al. 1994). A second investigation (Johnson et al. 1990a) identified 60 patients who were C. difficile carriers at the beginning of the study or became infected at some point during the nine weeks of the study. Two suffered from C. difficile diarrhea from the outset. Of the remaining 58, seven developed C. difficile diarrhea after 1 - 6 weeks of C. difficile-negative stools. Follow-up of the remaining 51 C. difficile carriers for a cumulative total of 69 weeks failed to identify any cases of diarrhea. This has been interpreted to indicate that if a patient is going to develop C. difficile diarrhea, it will occur shortly after having acquired the pathogen (Samore et al. 1994). Other authors failed to discern such a risk (Clabots et al. 1992, McFarland et al. 1990).

**Immunocompetence**

The role of the immune system in C. difficile carriage and disease is still unknown. Symptomatic carriers had significantly lower levels of C. difficile-specific serum IgA and IgM (p < 0.001) (Mulligan et al. 1993). *Clostridium difficile*-associated diarrhea lasting longer than two weeks was significantly associated (p = 0.001) with depressed serum IgG and fecal IgA antitoxin A titers (Warny et al. 1994). These same titers were significantly lower (p = 0.033) in adults with recurrent C. difficile-associated diarrhea compared to patients with non-recurrent C. difficile-associated diarrhea (Warny et al. 1994). Similarly, children with
chronic relapsing *C. difficile* colitis had significantly lower serum IgG antitoxin A levels than healthy children and adults (Leung et al. 1991). Furthermore, treatment with intravenous gamma globulin cured all the children of their symptoms and cleared their stool of *C. difficile* toxin (Leung et al. 1991). The above investigations complement the findings briefly covered in Section I of this review showing that animals can be protected from CDAD with passive or active immunization against *C. difficile*’s toxins. It has been proposed that a general decline with age in the immune response may account for the greater frequency of *C. difficile* disease observed with advanced age. Other studies question the notion (Johnson et al. 1992b) or the extent (Warny et al. 1994) of the systemic and mucosal antibody response to *C. difficile* toxin A.

Neither HIV infection nor AIDS has been associated with an increased risk of *C. difficile* acquisition (Samore et al. 1994) or carriage (McFarland et al. 1990). A case-control study of HIV-infected patients found that a diagnosis of AIDS or low CD4 counts were more common among *C. difficile*-associated diarrhea cases, but the difference was not statistically significant (p = 0.16 and p = 0.26, respectively). The overall rates of *C. difficile* diarrhea of 4.1/100 HIV-infected patient admissions likewise did not differ significantly (p = 0.12) from the 1.5 cases per 100 HIV negative patient admissions (Hutin et al. 1993).

Patients immunocompromised by malignancies and/or the associated chemotherapy have been reported to suffer from high rates of CDAD (Fainstein et al. 1981, Cudmore et al. 1982, Miller and Koornhof 1984). Patients with acute leukemia were found to have a significantly higher rate of *C. difficile* carriage than patients with other malignancies (Heard et al. 1988).
Length of Hospital Stay

A case-control study of HIV-infected patients found that the multivariate-adjusted odds ratio associated with each week of residence in the study ward was 3.6 (95% CI: 1-13) (Hutin et al. 1993). Length of hospitalization was associated with *C. difficile* acquisition ($R^2 = 0.96$) (Clabots et al. 1992).

McFarland et al. (1990) have looked at length of hospitalization while at the same time controlling for severity of underlying disease. In univariate analyses, both severity of underlying disease and length of hospitalization were significantly associated with *C. difficile* carriage. Yet in the multivariate analysis, only severity of underlying illness remained significantly associated. This result suggests that length of hospitalization may be a marker for severity of underlying illness.

Prior History of CDAD

Certainly, the recurrent nature of antibiotically treated CDAD is well documented. Part of the dramatic variation in reported recurrence rates stems from the use of different definitions of recurrence. To some authors the mere reappearance of *C. difficile* in the stool, with or without cytotoxin production, of previously culture-negative patients constitutes a recurrence; this has been termed a microscopic recurrence (Young et al. 1985, Johnson et al. 1989). To other authors it is the reappearance of symptoms that is the defining condition of a recurrence (Bartlett 1984, Fekety et al. 1989).

Recurrent CDAD could conceivably be due to reinfection of a highly susceptible person, as suggested by some of the systemic and mucosal immune system findings discussed above (Leung et al. 1991, Wanny et al. 1994). Alternately, it could represent the renewed overgrowth of *C. difficile* that
survived the treatment. The relative contributions of these two mechanisms to the rate of recurrent CDAD are not known.

Few studies have brought the full power of the microbiology laboratory to bear upon the issue of the nature of CDAD recurrences. Johnson and associates (1989) applied restriction endonuclease assay (REA) typing to isolates of C. difficile from patients with CDAD. Of the 11 patients who suffered microscopic relapses of CDAD, five (46%) turned out to be reinfected with other strains. These new strains were ascertained to have been nosocomially acquired.

In proposing reinfection as a common mechanism of recurrent CDAD, Johnson et al. (1989) suggest that even the recurrences showing the same REA pattern may represent reinfection, albeit with the same strain. However, the mean interval between episodes of REA-determined reinfections and relapses are 42.5 and 14.5 days respectively, suggesting that they represent two distinct phenomena. If all the recurrences with the same REA-typed strain were really caused by reinfections, no difference in the mean interval would be seen. In fact, the distributions of the two populations is not statistically distinct, insofar as the standard deviation around the reinfection mean is 39 days, thereby encompassing the relapse-associated mean.

Samore et al. (1993) found no evidence that reinfection is an important cause of recurrent CDAD. This group conducted REA typing on 15 patients with recurrent and 24 with non-recurrent cytotoxin-positive diarrhea. The REA types were identical in all six patients for whom isolates were typed both at the initial and the recurrent episode.

Issues of relapse versus reinfection aside, a prior history of C. difficile diarrhea was associated with a positive stool culture within 72 hours of admission to a hospital (OR=9.5; 95% CI:3.0-30.0); in fact, within this group, a culture was more likely to be positive if taken within 60 days of a prior episode
of *C. difficile* diarrhea (p = 0.03) (Samore et al. 1994). However, McFarland et al. (1989) found previous *C. difficile* infection to be associated with neither *C. difficile* carriage nor disease.

**Other Host Risk Factors**

Other risk factors for *C. difficile* carriage include H-2 blockers (Walker et al. 1993), anti-ulcer medications in general (Simor et al. 1993), stool softeners (mRR = 2.04; 95% CI: 1.14, 3.68) and antacids (mRR = 1.80; 95% CI: 1.02, 3.16) (McFarland et al. 1990). However, with regards to *C. difficile* diarrhea, only the use of stool softeners was a significant risk. In addition, McFarland and associates (1990) found gastrointestinal stimulants (mRR = 3.61; 95% CI: 1.08, 12.06) and enemas (mRR = 2.96; 95% CI: 1.36, 10.20) to be significant risks for *C. difficile* diarrhea. H-2 blockers and/or antacids were be significant risk factors on univariate analysis, but were subsequently found not to be significant upon logistic regression (Brown et al. 1990).

Feeding tubes were associated with carriage by an odds ratio of 6.5 (Simor et al. 1993), as were non-surgical gastrointestinal procedures, the most significant of which was a nasogastric intubation (Brown et al. 1990). While nasogastric intubation, endoscopy, enemas, and non-gastrointestinal surgery were age-adjusted risk factors for *C. difficile* carriage and diarrhea, none of these procedures remained significant after the multivariate analysis (McFarland et al. 1990). A retrospective case-control study of an epidemic found that gastrointestinal surgery carried a 7.9 (95% CI: 1.9, 35) greater risk of *C. difficile* diarrhea, though whether this risk was due to the surgery itself or to the perioperative antibiotics therapy is not clear (Thibault et al. 1991). Residence in an intensive care unit (Brown et al. 1990) was a risk factor for *C. difficile* diarrhea; in contrast, residence on a surgical ward was associated with a high risk of *C.
difficile acquisition (RR = 3.0; 95% CI: 1.1-8.5), even if no surgery was actually performed (Samore et al. 1994).

**Bacterial Risk Factors**

The debate over the association between toxin production and disease has raged for many years. What is clear is that identification of toxin-positive stools is the most specific method for the diagnosis of CDAD (Gerding and Brazier 1993), and that the sensitivity of the method increases with the severity of the clinical manifestations (Table II-4). What is not clear, however, are the relative roles that toxins and other strain characteristics play in the pathogenesis of the disease.

**Bacterial Toxins and Disease**

As shown in table II-4, cytotoxin is more commonly detected in the stools of patients with PMC than in those with AAD. While this association strongly suggests a role for the cytotoxin in disease, other evidence indicates that the cytotoxin is not a sufficient cause of disease. Healthy infants have been shown to harbor concentrations of C. difficile and its cytotoxin comparable to concentrations seen in adults with PMC (Rolfe 1988). Even within the adult population, the titers of cytotoxin isolated from the stools of asymptomatic recipients of antibiotics did not differ markedly from that detected in patients with AAPMC or AAC (George et al. 1982). Neither the presence of cytotoxin or endotoxin in stools, nor the titers elaborated *in vitro* by isolates, was statistically associated with symptomatic carriage (McFarland et al. 1991). Indeed, McFarland and associates (1991) calculated the positive predictive value of C. difficile diarrhea in stool cytotoxin to be 44%, while it was 39% in *in vitro* production. Furthermore, cytotoxin has been demonstrated in the stools of
patients rendered asymptomatic after vancomycin therapy (Peterson and Kelly 1993). Thus, neither the presence nor the concentration of toxins is sufficient to account for CDAD; either host- or strain-specific factors must be sought to explain the variability in the effects of C. difficile's toxins.

Consistent with this conclusion, McFarland et al. (1991) found that among high-risk patients, the isolates associated with high titers of cytotoxin or enterotoxin production in vitro were more common in symptomatic patients (p = 0.12 and p = 0.06, respectively); among low-risk patients, the relationship was reversed, with significantly more asymptomatic patients having high-titer production of cytotoxin and enterotoxin (p = 0.02 and 0.01, respectively). The patient risk index was calculated based on a multivariate analysis of risk factors for CDAD (McFarland et al. 1990).

Bacterial Strains and Disease

Not all strains of C. difficile elaborate toxins. Moreover, not all toxigenic strains produce the same amount of toxin in vitro. In fact, the same immunoblot-identified strain, and even the same isolate, can produce variable titers of toxin under identical conditions (McFarland et al. 1991).

Four prospective cohort studies have examined the role of bacterial factors in C. difficile carriage and disease in adults (Clabots et al. 1992, McFarland et al. 1991, Johnson et al. 1990a, Tabaqchali 1990). Unfortunately, the results of the typing cannot be compared because three of the four studies used different typing methods.

Two studies found no association between isolate type and disease (McFarland et al. 1991, Clabots et al. 1992). These findings are consistent with the demonstration that asymptomatic carriers are the source of many nosocomial acquisitions leading to disease (McFarland et al. 1989). In contrast, two other
studies (Johnson et al. 1990a, Tabaqchali 1990) found that a significant risk was associated with particular strains. However, among the patients who were admitted to the study ward during the investigation by Tabaqchali (1990), the strain most commonly associated with disease was significantly more common in patients who had acquired *C. difficile* during the study period (*p* < 0.005). In the study by Johnson and team (1990a), 88% of the isolates of the epidemic strain were from patients in the surgery ward. Because both recent acquisition and residence in a surgery ward have been identified as possible independent risk factors for *C. difficile* diarrhea (Samore et al. 1994), conclusions regarding the association between strain type and disease based on these two studies are questionable.

These considerations also make it hard to determine whether there is a strain-specific component to epidemicity. Tabaqchali (1990) pointed out that while uncolonized patients admitted to the study ward acquired the epidemic strain, which was already present on the ward at the onset of the study, other strains subsequently introduced by new admissions failed to lead to large-scale acquisition in subsequent uncolonized admissions.

Where an "epidemic" strain was identified, the titers of inducible cytotoxin either were not measured (Johnson et al. 1990a, Clabots et al. 1992) or were variable across different isolates of the same strain (Tabaqchali 1990). Tabaqchali (1990), however, reported higher titers in symptomatic patients, though the researchers do not provide any measure of significance.

**Future Research**

Published incidences of CDAD five years ago ranged from 0.01 to 10% of hospital admissions (Lyerly et al. 1988). Moreover, the National Nosocomial Infections Surveillance (NNIS) indicates that the incidence of nosocomial *C.
*difficile* diarrhea in its reporting hospitals is increasing (Samore et al. 1994). This increase may be an artifact due to the increased awareness among health care providers that *C. difficile* is the most common identifiable cause of nosocomial diarrhea and that it is associated with a relatively high rate of morbidity and mortality. Alternately, it could reflect a true increase, due to an increase in any of the risk factors discussed above or one as yet unidentified.

Surprisingly few studies have even discussed the mortality rate associated with CDAD (Monti et al. 1992, Peterson and Kelly 1993, Eriksson and Aronsson 1989, Thomas et al. 1990). One study has demonstrated that the mortality rate within six weeks of a CDAD diagnosis in the elderly is three times that in controls matched for sex, age, and underlying disease (*p* < 0.001); thromboembolic complications are the salient cause of death, perhaps due to prolonged immobility resulting from CDAD (Eriksson and Aronsson 1989). An investigation of elderly nursing home residents receiving antibiotics found that the 12-month mortality rate was significantly higher for those patients who carried *C. difficile*, whether or not they were symptomatic (Thomas et al. 1990).

Given the magnitude of the problem, infection-control and prophylactic measures are certainly desirable. For this reason careful analysis of the risk factors associated with acquisition, carriage, and disease is important. The feasibility and efficacy of targeting each one of these states should be considered.

One surprise in the literature that needs further attention is the suggestion by Samore et al. (1994) that asymptomatic carriers are far less likely to develop disease than patients who have newly acquired the bacterium. This hypothesis has tremendous public health implications, insofar as it dramatically reduces the number of people who would need to be considered eligible for prophylaxis. Furthermore, given that attempts to eradicate asymptomatic carriage with
antibiotics actually prolonged carriage (Johnson et al. 1992a), asymptomatic carriage should not be targeted by the methods currently available.

Asymptomatic carriers would not become an unimportant entity, however, since they have been shown to be a significant source of nosocomial contamination; patients infected by exposure to an asymptomatic roommate are at risk of developing disease. Although the case is flimsy for the contention that recent acquisition is a risk factor for *C. difficile* diarrhea, it is nonetheless of crucial importance insofar as prophylaxis and infection control measures are concerned. A prospective study should be designed specifically to test this hypothesis.

McFarland and associates' (1990) investigation of the risk factors associated with the acquisition of *C. difficile* was rigorous and large enough to achieve significant power. Nevertheless, it needs to be repeated, both to confirm the researchers' findings and to extend them to a setting more characteristic of endemic *C. difficile* acquisition.

The lack of a standard typing system for *C. difficile* isolates is a severe impediment to resolving the issue of whether there are strains specifically associated with epidemics or disease. At least some of the typing systems currently employed are consistent with individual strains that elaborate variable titers of toxin under the same conditions. Clearly, these typing schemes are not sufficiently specific to elucidate this important epidemiological facet of *C. difficile*.

The drug of choice for treating symptomatic CDAD is vancomycin. While this drug is much more expensive than comparably-effective metronidazole, there are reported cases of bacterial resistance to the latter drug. Because of the dreaded complications of CDAD and the delay of up to one week required to determine the antibiotic susceptibility of an isolate, many physicians empirically treat for CDAD with vancomycin. This behavior poses a potentially serious
problem, since vancomycin remains the last resort in treating enterococci that have become resistant to all other drugs (Peterson and Kelly 1993).
<table>
<thead>
<tr>
<th>Test (Toxin)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture (fox b)</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
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<tr>
<td>Clinical parameters</td>
<td>69</td>
<td>96</td>
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<td>96</td>
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<tr>
<td>Tissue culture (fox b)</td>
<td>69</td>
<td>96</td>
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<td>Clinical parameters</td>
<td>69</td>
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<td>Tissue culture (fox b)</td>
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<td>96</td>
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<tr>
<td>Clinical parameters</td>
<td>69</td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Labs (w/o culture)</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
</tbody>
</table>

Table II. Characteristics of Immunoassay Tests for the Detection of C. difficile Toxins A or B
Table II-2. Typing schemes for *Clostridium difficile*

Susceptibility Patterns
- Antibiotic sensitivity (antibiograms)
- Bacteriophage, bacteriocin, and other sensitivity markers

Electrophoretic Protein Patterns on SDS-PAGE
- Coomassie staining
- Silver staining
- $^{35}$S-methionine labelled

Immunologic Markers
- Crossed immunoelectrophoresis (CIE)
- Immunoblotting (IB)
- Agglutination (serogrouping)

Genetic Markers
- Plasmid analysis
- Restriction endonuclease analysis (REA)
- Ribosomal RNA probes

Molecular Probes
- Specific proteins
- Toxins
- Antibiotic-resistance genes
Table II-3a. Results of prospective surveys of the carriage and acquisition rates of *Clostridium difficile* in different patient populations in the absence of outbreaks of *Clostridium difficile*-associated diarrhea.

<table>
<thead>
<tr>
<th>Ward</th>
<th># patients (specimens)</th>
<th>Months of study</th>
<th>Isolation rate (%)</th>
<th>Carriage rate (%)</th>
<th>Acquisition rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>116 (292)</td>
<td>3</td>
<td>8.2</td>
<td>5.8</td>
<td>2.3</td>
<td>Tabaqchali 1992</td>
</tr>
<tr>
<td>Geriatric</td>
<td>68 (243)</td>
<td>6</td>
<td>10.3</td>
<td>5.9</td>
<td>4.4</td>
<td>Tabaqchali 1992</td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>116 (292)</td>
<td>3</td>
<td>11.2</td>
<td>8.6</td>
<td>2.6</td>
<td>Tabaqchali 1992</td>
</tr>
<tr>
<td>General medical</td>
<td>438</td>
<td>9</td>
<td>3.9</td>
<td>1.4</td>
<td>2.5</td>
<td>Heard 1986</td>
</tr>
<tr>
<td>General medical</td>
<td>428</td>
<td>11</td>
<td>29</td>
<td>7</td>
<td>21</td>
<td>McFariand 1989</td>
</tr>
</tbody>
</table>

Table II-3b. Results of prospective surveys of the carriage and acquisition rates of *Clostridium difficile* in different patient populations in the during outbreaks of *Clostridium difficile*-associated diarrhea.

<table>
<thead>
<tr>
<th>Ward</th>
<th># patients (specimens)</th>
<th>Months of study</th>
<th>Isolation rate (%)</th>
<th>Carriage rate (%)</th>
<th>Acquisition rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological malignancy</td>
<td>135 (538)</td>
<td>6</td>
<td>36.5</td>
<td>6.6</td>
<td>21.5</td>
<td>Heard 1986</td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>62</td>
<td>6</td>
<td>39.9</td>
<td>7.7</td>
<td>32.2</td>
<td>Delmee 1987</td>
</tr>
<tr>
<td>Geriatric</td>
<td>54 (258)</td>
<td>6</td>
<td>25.9</td>
<td>11.1</td>
<td>14.8</td>
<td>Tabaqchali 1992</td>
</tr>
</tbody>
</table>

from Tabaqchali 1992
Table II-4. Results of tissue culture assays for *C. difficile* toxin and stool cultures for *C. difficile*

<table>
<thead>
<tr>
<th>Patient/disease category</th>
<th>Percentage of patients with positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toxin (Cell culture)</td>
</tr>
<tr>
<td>Antibiotic-associated PMC</td>
<td>95-100</td>
</tr>
<tr>
<td>Antibiotic-associated diarrhea w/o confirmed PMC</td>
<td>15-25</td>
</tr>
<tr>
<td>Antibiotic exposure w/o diarrhea</td>
<td>2-8</td>
</tr>
<tr>
<td>Gastrointestinal disease unrelated to antimicrobial exposure</td>
<td>0-0.5</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>0</td>
</tr>
<tr>
<td>Healthy neonates</td>
<td>5-63</td>
</tr>
<tr>
<td></td>
<td>Stool culture</td>
</tr>
<tr>
<td></td>
<td>95-100</td>
</tr>
<tr>
<td></td>
<td>25-50</td>
</tr>
<tr>
<td></td>
<td>10-25</td>
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<td></td>
<td>3-5</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5-70</td>
</tr>
</tbody>
</table>

from Bartlett 1992
III--Intestinal Microflora

A New Model

The epidemiologic features of *C. difficile*, most notably the high risk associated with antimicrobial therapy, calls for a new paradigm of disease that may generate a new approach to treating and preventing CDAD. Rather than assuming an adversarial relationship with all microbes, we must come to see disease as a disruption of the normal balance that exists between human beings and the microorganisms sharing their external environment.

All surfaces of the human body that are topologically contiguous with the outside environment are colonized by bacteria (ignoring the fact that the fallopian tubes are open to the peritoneum). The dominant view of the host-parasite interaction maintains that over time the host and its bacterial parasite evolve a dynamic state of harmony. This harmony explains why the bacteria that regularly colonize human skin and mucus membranes rarely cause disease in the ecological niche they inhabit.

The antibiotics that play such an important role in CDAD pathogenesis are believed to induce disease precisely because they disrupt this harmonious interaction in the human intestines. Because CDAD is a consequence of failing to consider the entire biological context in which the antimicrobics act, it is instructive to take a brief foray into the intestinal microflora, its composition, the role it plays in health and disease, and the factors that affect it, before turning to the *C. difficile*-specific literature.
Intestinal Microflora in Health and Disease

Ecology and Composition

Microorganisms may associate with the intestines in a variety of manners. Bacteria may "colonize" the gastrointestinal tract, a term which implies that the population is stably maintained over time. Ingested bacteria failing to colonize may nevertheless persist in the intestines for a short while in what may be called a "transient" state. Bacterial strains not normally found in the intestines are "invaders", and their colonization of the bowel is called "infection."

The term indigenous microflora refers to the complex mixture of bacterial populations that colonize the intestinal tract of human beings in the absence of medical interventions or pathological processes (Freter 1992). Complex it is, as over 400 species of bacteria representing more than 30 genera have been identified in the intestines of a single healthy human being (Wilson 1993).

While the composition of the indigenous microflora differs among people, it is reported to be quite stable over time (Gorbach et al. 1967, Holdeman et al. 1976). Nevertheless, many factors can perturb the composition of this flora, some of which will be discussed. In fact, it is the disruption of the flora by antibiotics that motivates this discussion. The goal is to identify means to restore the flora.

The complexity of the indigenous microflora refers not only to the number of different species but also to the microenvironments present within the intestinal ecosystem. The intestines offer anatomically, histologically, chemically, and physically distinct niches for bacteria, the interplay of which must be taken into consideration to contravene the effects of antibiotics. For the purposes of the following discussion bacteria may be placed in one of three categories depending upon their tolerance of molecular oxygen: aerobic, anaerobic, or facultative anaerobic. This last group will use oxygen if it is available but functions fine under anaerobic conditions.
The stomach is relatively sterile due to its gastric acidity, active peristalsis, and relatively high oxidation-reduction potential. During eating, when the acidity of the gastric juices are buffered by food and large numbers of organisms are introduced in either the food or washed down from the oral cavity, the stomach can contain $10^5$ organisms per milliliter. Within a few hours, however, the counts return to virtually undetectable levels (Hentges 1993). Gram-positive facultative bacteria such as *Streptococcus*, *Staphylococcus*, and *Lactobacillus* are the most commonly isolated organisms (Goldin 1990).

The small intestine has a flora that reflects its position as a bridge between the relatively sterile Gram-positive aerobe-dominated stomach to the teeming Gram-negative anaerobe-dominated colon. The composition of the flora, therefore, will depend upon the location sampled. Chemical factors constraining bacterial populations in the small bowel include the bile acids and the pancreatic juices.

Crossing the ileocecal sphincter separating the small bowel from the large bowel or colon, bacterial counts increase five to six orders of magnitude, to $10^{11}$ or $10^{12}$ microorganisms per milliliter of fecal material (Goldin 1990). So numerous are the colonic microorganisms that one-third of the dry weight of fecal matter is due to viable bacteria (Goldin 1990).

In the colon, Gram-negative genera are significantly more common than Gram-positive bacteria (Simon and Gorbach 1984), and anaerobes outnumber aerobes by two to four log$_{10}$ units (Hentges 1993). This last fact is clinically relevant because of the role indigenous intestinal anaerobes play in human health and disease.
Morphological Effects

Studies in germ-free animals have revealed that the intestinal microflora have far-ranging effects on both the histology and physiology of the intestines themselves. Morphological differences are present throughout all layers of the intestines. The wall is thinner and less cellular. The villi are also thinner and more pointed, while the crypts of Lieberkuhn are shallower. The most striking effect seen is the 10-fold enlargement of the cecum, which rapidly returns to normal size when the the animals are colonized. As expected, the lamina propria of germ-free animals show very little development associated with mucosal immunity, namely Peyer’s patches, germinal centers, and lymphocyte and macrophage infiltration of the stroma (Simon and Gorbach 1984). Gastrointestinal transit time and enterocyte turnover are significantly decreased (Savage 1986). The intraluminal pH is more alkaline, and the oxidation-reduction potential (Eh) is more positive (Simon and Gorbach 1984).

Metabolic Actions

One clinically important metabolic action of the intestinal microflora involves the metabolism of ammonia. Most human intestinal bacteria will use ammonia as a source of nitrogen for protein synthesis (Vince 1986), and it has traditionally been held that ammonia is their preferred source (Hentges 1993). Fecal ammonia production, in turn, is largely due to bacterial metabolism of urea and nonurea sources such as peptides; germ-free animals have much lower concentrations of ammonia in their intestines (Vince 1986).

The metabolism of ammonia is significant in portal-systemic encephalopathy (PSE), a condition in which circulatory changes associated with liver disease enable absorbed intestinal toxins to bypass the liver and reach the brain, compromising cerebral function. Ammonia is the toxin most often
implicated, and the major therapeutic interventions address the central role of bacteria in the metabolism of fecal ammonia.

One element of PSE treatment is to give lactulose, a synthetic disaccharide that provides effective control in 70 to 85% of chronic PSE cases. This carbohydrate is not metabolized by human disaccharidases but instead passes unchanged through the small intestine to the colon, where it influences the metabolic activity of the intestinal bacteria in such a way as to decreases fecal ammonia levels. In the absence of carbohydrates for an energy source, intestinal bacteria metabolize nitrogen sources such as the proteins in sloughed intestinal epithelium, which leads to increased ammonia production. Lactulose, by providing an alternative energy source, inhibits this process. In addition, it provides the carbon skeletons required for the incorporation of ammonia nitrogen into bacterial protoplasm.

Other mechanisms proposed for the action of lactulose include: a cathartic effect which, by decreasing bowel transit time, will reduce the time available for bacteria to metabolize nitrogenous substrates; a stimulatory effect on acidophilic bacteria and a concomitant inhibitory effect on ammonia-producing species; a pH-lowering effect secondary to its fermentation. A pH below 5.0 will have the dual effect of markedly reducing bacterial synthesis of ammonia while at the same time converting ammonia to the ammonium ion, which is much less readily absorbed (Vince 1986).

Whichever mechanism is ultimately responsible, the use of lactulose is significant conceptually as well as clinically, insofar as it accords a prominent place to the practice of treating a medical condition by manipulating the metabolic activity of intestinal microbes.

The drug salicylazosulfapyridine (Azulfidine) provides another example of how the metabolic action of intestinal bacterial enzymes can be enlisted for
therapeutic effect. This drug represents the fusion via an azo bond of two pharmacologically distinct moieties, an antibiotic and an anti-inflammatory salicylate. Because human beings do not have azo-reducing enzymes on the intestinal mucosa, this compound passes unmodified through the small intestine, and would emerge unchanged in the feces were it not for the azoreductases of colonic bacteria. These bacteria split the drug into its two functional parts, resulting in high concentrations of anti-inflammatory salicylates in the colon which are useful in treating ulcerative colitis (Goldin 1990).

While the azoreductases are beneficial in this drug-delivery scheme, they, along with other intestinal bacterial enzymes such as nitroreductases and β-glucuronidases, have been implicated in carcinogenesis (Goldin 1990). The liver detoxifies many compounds by conjugating them with glucuronic acid to form glucuronides, which are then excreted into the intestines via the bile. Bacterial glucuronidases hydrolyze the bond, regenerating the toxin which may then be either reabsorbed by the intestines or act locally. The azo- and nitroreductases convert azo and nitro compounds to aromatic amines, generating highly reactive intermediates and end products that are known mutagens and carcinogens. Both azo dyes and nitro compounds are used as additives in the food industry.

Ample evidence points to diet as an important factor regulating the expression of these bacterial enzymatic activities. Diets can have an effect on the expression of bacterial enzymes or on the composition of the intestinal flora itself (Fuchs et al. 1976, Lhuillary et al. 1982, Hill 1986). Specifically, diets low in fat and high in fiber are associated with low activities, while the opposite is true for high-fat, low-fiber diets (Goldin and Gorbach 1984). Epidemiologic studies of colon cancer have also found an association between high-fat, low-fiber diets and colon cancer (Reddy 1980, Armstrong and Doll 1975).
The same diet has been linked to breast cancer (Gorbach 1984). Estrogens have long been implicated in breast cancer. Now, the role of intestinal bacteria in the metabolism of estrogens is multiple. Bacterial enzymes can either oxidize or reduce estrogens and androgens, depending on such factors as the concentration of bacteria and the amount of oxygen available to them (Gorbach 1984). Some of these products have enhanced biological activity (Bokkenheuser and Winter 1983).

Circulating levels of estrogens are maintained by enterohepatic circulation (EHC), a process whereby compounds inactivated by hepatic glucuronidation and excreted in the bile are metabolized by intestinal bacteria to an active form that is reabsorbed by the body. Factors that increase intestinal $\beta$–glucuronidase activity, such as high-fat and low-fiber diets, will consequently decrease the amount of estrogens excreted. Low-fat and high-fiber diets, as might be expected, increase fecal elimination of estrogens (Gorbach 1984).

Antibiotics, by reducing the population of colonic bacteria, likewise increase excretion of estrogens. This effect explains a pearl of medical lore: antibiotics can interfere with the effectiveness of oral contraceptives. Disruption of the intestinal microflora by antibiotics such as rifampin, ampicillin, and tetracycline can significantly compromise the effectiveness of the contraceptives (Thompson 1986).

EHC plays an integral part in the pharmacokinetics of many other drugs, insofar as hepatic glucuronidation is an important method whereby the body rids itself of potentially toxic compounds. Intestinal bacteria can change the pharmacokinetics of drugs by other means as well. Digoxin, a powerful heart medication with a relatively narrow therapeutic index, is metabolized by a glycosidase of *Eubacterium lentum* (Goldin 1990). Antibiotics that affect this
bacterium could lead to a potentially fatal increase in the bioavailability of a
given dose of digoxin.

Steroids represent another class of compounds upon which bacterial
enzymes act in clinically significant ways. Consumers of the high-fat, low-fiber
diet mentioned above may be somewhat more kindly disposed towards their
intestinal bacteria when they learn that some strains of anaerobic intestinal
bacteria convert cholesterol to a non-absorbable form called coprostanol
(Bokkenheuser 1993). Strains of *E. lentum* convert a potentially carcinogenic
product of human cholesterol metabolism, tetrahydrodeoxyxocorticosterone, to the
noncarcinogenic pregnalolone (Thompson 1986).

**Disease Resistance**

The notion that the intestinal flora could protect the host against infection
was first proposed in 1916 (Hentges 1983). Antagonistic interactions between
coliform bacteria were observed in 1925. A product secreted by intestinal
bacteria that hindered the multiplication of other bacteria was identified in 1947.

Over the years, many labels have been applied to this protection against
infection, including "bacterial antagonism" (Freter 1956), "bacterial interference"
(Dubos 1963), "barrier effect" (Ducluzeau et al. 1970), and "competitive exclusion"
(LLoyd et al. 1977). However, "colonization resistance" is the most widely used
term (van der Waaij et al. 1971).

The evidence supporting the existence of colonization resistance is
massive. It includes such *in vivo* methodological strategies as demonstrating an
increased susceptibility to colonization in gnotobiotic animals; restoring resistance
to colonization by adding simple, complex, or complete mixtures of intestinal
flora; reproducing the gnotobiotic studies in animals whose intestinal microflora
has been disrupted with a variety of antibiotics. Unravelling the mechanisms
behind the microbial interactions responsible for colonization resistance has additionally required the use of various *in vitro* techniques.

Methodological Issues

Methods traditionally used to test the interactions between at most a few bacteria *in vitro* have been used to characterize bacterial interactions deemed significant in colonization resistance. These methods include seeking evidence of growth inhibition in broth cultures, detecting inhibitory substances produced in liquid media by filtration or diffusion across semi-permeable membranes, and identifying diffusible inhibitory substances produced in solid media by the appearance of inhibition zones or cross streaks.

The value of these methods is limited to the most exploratory of preliminary studies because they fail to reproduce even the most basic features of the intestinal environment. For example, it would be misleading to extrapolate to the intestines the finding that lactobacilli in culture destroy Enterobacteriaceae due to their ability to ferment sugars to form lactic acid, for such an extension ignores the fact that the concentrations of these sugars are extremely low in the colon where the Enterobacteriaceae are common in addition to the fact that lactic acid produced in the intestines is readily absorbed across the intestinal mucosa (Raibaud 1992).

Furthermore, much is made about the isolation of antibacterial protein compounds such as colicins and bacteriocins. Yet the antagonism between colicinogenic and colicin-sensitive strains detected *in vitro* was not observed in germ-free animal models (Ikari et al. 1969). What's more, bacteriocins have never been detected *in vivo* (Raibaud 1992).

The most successful *in vitro* model for studying these interactions is the anaerobic continuous-flow (CF) culture. As the name implies, the success of this
model derives largely from having incorporated a key physical feature of the intestines: the continuous flow of luminal contents. The dense and complex populations of anaerobic bacteria that develop along the walls of the culture apparatus reproduces the numerical relationships among the complex flora observed in the large intestine (Freter 1992). Further corroboration of its validity comes from the demonstration that the concentration and composition of the metabolic end-products from CF culture are similar to those found in vivo (Freter et al. 1983).

An indispensable advance for studying bacterial interactions was the development of gnotobiotic animal models, germ-free animals that have been inoculated with one or more known bacterial strains (Raibaud 1992). Such animals have provided the basis for much of the evidence in support of colonization resistance and the mechanisms accounting for it.

Rodents are the least expensive and most convenient germ-free models. These animals make appropriate models insofar as their flora, like humans', is predominantly anaeorobic, complex, and stable (Wilson 1993). Their flora, however, contains species not found in humans, which may alter the details of specific interactions. Human intestinal flora can be successfully transferred to germ-free mice or rats, resulting in what is termed a polyassociated animal (Raibaud 1992). However, caution must obviously be exercised in drawing conclusions from such polyassociated models.

There is concordance with regards to the ecological succession of the predominant bacteria as the germ-free animals and human infants develop an intestinal flora. Both hamsters and human infants are initially colonized by C. difficile, but as the intestinal microflora reaches its climax stage, C. difficile disappears from both (Wilson 1993).
Even with these sophisticated models, there are important technical issues to bear in mind. Gnotobiotic animals differ from conventional animals with respect to their intestinal contents, peristalsis rate, and local and systemic immune mechanisms (Freter and Abrams 1992). These factors are believed to be crucial to the interactions present in the intestines.

Furthermore, these models still suffer some of the shortcomings of the traditional in vitro techniques. For, while gnotobiotic models remedy many of the deficiencies arising from ignoring the role of the host in, say, nutrient bioavailability, the problems associated with a drastically simplified intestinal microflora still remain. Thus, in contrast to the above-mentioned inability to detect colicin-based antagonism in an animal model, a bacterium secreting a bacitracin-like antibiotic was documented to prevent the growth of sensitive strains of C. perfringens in gnotobiotic mice (Ducluzeau et al. 1976). Antibiotic production disappeared, however, if a strain of Lactobacillus was added to the intestinal environment.

Colonization Resistance in Animal Models

By the end of the 1950s there was already considerable supportive evidence for colonization resistance. A natural experiment in the early 1950s gave an effective demonstration of this principle. An epidemic of Salmonella enteritidis ravaging a population of stock mice was successfully controlled when the antibiotic oxytetracycline was added to their drinking water. Guinea pigs that drank the treated water, however, began succumbing to infection with a strain of Proteus that was resistant to the antibiotic. Removing the antibiotic from the water terminated this second epidemic (Meynell 1955).

Around the same time, researchers began examining the role of the intestinal flora in controlled experiments. Oral antibiotics covering a broad
spectrum of bacteria made guinea pigs and mice vulnerable to infection with antibiotic-resistant strains of two important human intestinal pathogens, *Salmonella flexneri* and *Vibrio cholerae* (Freter 1955, 1956). The dose of *S. enteritidis* necessary to infect 50% of the mice challenged with the bacterium (ID$_{50}$) was shown to be 10,000 times lower in mice pretreated with the antibiotic streptomycin (Bohnhoff and Miller 1962). Demonstrating that the streptomycin virtually eliminated the Gram-negative bacilli from the intestinal tract of the susceptible mice (Miller and Bohnhoff 1963) supported the notion that disruption of the flora, and not some antibiotic-induced expression of *Salmonella* virulence factors, was responsible for the susceptibility.

These studies were complemented with experiments comparing gnotobiotic animals to their conventionally reared counterparts. The intestines of gnotobiotic animals had markedly higher counts of *S. enteritidis* (Nardi et al. 1990), *Shigella flexneri* (Maier et al. 1972), or *Campylobacter jejuni* (Jesudason et al. 1989) following oral inoculation with these three leading causes of bacillary dysentery. Furthermore, after intragastric challenge with *Salmonella typhimurium*, gnotobiotic mice colonized with indigenous strains of *Lactobacillus*, *Bacteroides*, and *Clostridium* had markedly lower intestinal titers of the challenge strain than did germ-free controls (Roach and Tannock 1979).

Even so, these colonized gnotobiotic mice were still more susceptible to infection than conventionally reared mice. In fact, it has not proven possible to define a subset of the indigenous microflora capable of fully reproducing the level of colonization resistance obtained by repopulating gnotobiotic mice with whole flora, even when repopulating with a mixture containing as many as 95 (Freter and Abrams 1972) or 110 (Koopman et al. 1981) different strains of anaerobic bacteria.
The fact that the repopulating strains used were anaerobic reflects the disputed-yet-long-held notion that it is the anaerobic component of the bacterial flora that is most responsible for maintaining resistance to infection (Bonhoff et al. 1962, van der Waaij et al. 1971 & 1974). Other authors have concluded that both strict and facultative anaerobes are important (Freter and Abrams 1972, Koopman et al. 1981). One study, in human beings, concluded that colonization resistance is not related to the anaerobic microflora (Gorbach et al. 1988). The issue is an important one, since some physicians who are cognizant of colonization resistance are reluctant to prescribe prophylactic antibiotic regimens capable of disrupting the population they deem responsible for maintaining protection against infection (Gorbach et al. 1988).

Colonization Resistance in Human Beings

In fact, not only is the bacterial population responsible for colonization resistance still undefined, the phenomenon has yet to be conclusively demonstrated in human beings. Three modestly controlled experiments in humans claim to corroborate the existence of colonization resistance in human beings (Buck and Cooke 1969, Krause et al. 1969, Gorbach et al. 1988). Buck and Cooke (1969) fed three healthy volunteers Pseudomonas aeruginosa and examined their stools for the ingested pathogen. One volunteer took a four-day course of ampicillin on three occasions prior to receiving the P. aeruginosa challenge; on one of these occasions the stool samples showed a higher fecal titer of the bacterium than the volunteer had ingested. On this and the other two occasions, it took the volunteer considerably longer than the two other volunteers for his stools to be cleared of P. aeruginosa. This constellation of findings was taken to indicate that the ampicillin regimen had disrupted the volunteer's colonization resistance.
Another trial (Gorbach et al. 1988) fed four groups of four healthy volunteers a different antibiotic for nine days; a control group took no antibiotics. The groups were then challenged with well-characterized strains of *E. coli* and *P. aeruginosa*. While control and test groups did not differ with respect to the fed bacterial strains, seven of the 16 antibiotic recipients became colonized with Gram-negative bacilli from the environmental at the same time that none of the control group did.

Indirect evidence for colonization resistance in human beings comes from several studies of salmonella enteritis following antibiotic administration. The first case ever reported (Black et al. 1960) describes a patient who was given oxytetracycline as prophylaxis prior to surgery on his hand (Finger and Wood 1955). His stools grew out *Salmonella muenchen*, which he is believed to have been carrying prior to surgery. Another case study reported the onset of grossly bloody diarrhea in an asymptomatic carrier of *Salmonella typhimurium* three days after beginning an ampicillin regimen (Rosenthal 1969). Treating patients suffering from acute salmonella enteritis with the antibiotics chloramphenicol or ampicillin markedly increased the duration of postconvalescent excretion of the organism (Aserkoff and Bennett 1969).

Somewhat sounder yet still indirect evidence comes from a macabre study on human volunteers that, in order to determine the effectiveness of a typhoid vaccine, sought to establish the infectious dose that would lead to disease in 50% of healthy volunteers (ID₅₀) challenged with *Salmonella typhi*, the agent responsible for typhoid fever (Hornick et al. 1970). Clinically detectable typhoid fever developed in one of four volunteers treated with the antibiotic streptomycin before receiving the *Salmonella* challenge; in contrast, none of the 14 controls developed clinical illness following a similar challenge.
Colonization Resistance—Mechanisms

Three mutually compatible mechanisms are routinely invoked to explain colonization resistance: competition for nutrients, competition for adhesion sites, and the release of metabolites toxic to other bacteria. These mechanisms apply to colonization resistance of the intestines as well as of the urogenital tract.

Attesting to the importance of competition for nutrients, inoculating a filtrate of a CF culture of mouse cecal flora with *E. coli*, *Fusobacteria*, *Eubacteria* (Freter et al. 1983) or *C. difficile* (Wilson 1988) failed to elicit growth of these bacteria; the medium lacked an adequate source of carbohydrate, which limited the growth of the would-be "invaders" but not the established flora. Nutrient sources in the intestines include those derived from the diet itself, host-derived substrates such as the mucus gel overlying the epithelium of the intestinal tract and sloughed epithelium cells themselves, metabolites from microorganisms acting on the above substrates, and finally, the protoplasm of dead microorganisms.

Theoretically, if a microorganism does not multiply faster than the rate at which it is washed out of a given region of the intestines by the movement of the lumenal contents, it cannot colonize that region. Competition for nutrients slows the rate of reproduction, so that only those microorganisms most able to compete will succeed in colonizing the intestines. Under standard CF culture conditions, *C. difficile* multiplies too slowly to colonize the apparatus. Adding a fermentable carbohydrate to the medium increases its growth rate to achieve colonization of the CF culture (Wilson 1993).

Microorganisms unable to compete for nutrients, however, can circumvent their disadvantage by attaching to the mucosa, thereby reducing the rate at which they are washed out (Freter et al. 1983). Mucosal surfaces of rodents treated with antibiotics bound challenging doses of *C. albicans* (Kennedy
and Volz 1985) or enterotoxigenic *E. coli* (ETEC) and *Shigella sonnei* (Pongpech et al. 1989) significantly more than the mucosa of rodents not exposed to antibiotics.

Toxic metabolites are credited with a large role in excluding exogenous bacteria from the intestines. Hydrogen sulfide, produced in large quantities by the colonic flora, suppresses the growth of *E. coli* in CF culture systems (Freter et al. 1983); it may do so by diminishing its ability to compete for nutrients (Wilson 1993). Secondary bile acids, bacterial metabolites of the primary bile acids synthesized by the liver, suppress many intestinal Gram-positive and Gram-negative bacteria (Floch et al. 1972).

By far the most thoroughly studied metabolite, however, are the short-chain volatile fatty acids, which include formic, acetic, propionic, and butyric acids. The evidence comes from *in vitro* suppression (Meynell 1963, Bohnhoff et al. 1964), *in vivo* results (Maier et al. 1972, Que and Hentges 1985), and clinical studies (Tazume et al. 1993), and a host of correlations between these three classes of information (Hentges 1992). The findings, while predominantly supportive of a major role for volatile fatty acids, are nevertheless not unanimous (Freter and Abrams 1972, Koopman et al. 1981).

**Alternate Mechanisms of Disease Resistance**

Colonization resistance, though certainly the most widely studied mechanism whereby microorganisms inhabiting the intestines are thought to protect against disease, is not the only conceivable way. While colonization is a necessary first step in the pathogenesis of most intestinal pathogens, it is not sufficient. Toxin production is often a key to disease causation. Thus, there are several points down-stream from colonization at which the intestinal microflora can prevent disease.
Conceivably, the components of the microflora could suppress elaboration of certain toxins. Alternately, the association of the toxin with its receptor could be hindered, by modifying either the toxin or the receptor. Finally, the effect of the toxin on the host cell could be antagonized. The latter two mechanisms have been proposed to account for the protective effects of a yeast, *S. boulardii*, currently being investigated for potential use against CDAD and cholera, respectively. The literature is discussed in more detail in Section IV.

**Factors Affecting Composition and Metabolism**

Diet, age, stress, physicochemical conditions, the immune system, and the composition of the microflora itself have all been proposed as elements affecting the metabolic activity of the intestinal microflora. These factors exert their effects either indirectly, by changing the composition of the flora, or directly, by altering the expression or activity of enzymes.

**Methodological Considerations**

Much of the research published on the effects of these factors relies on analysis of fecal flora and enzymatic activity. This is true because stool specimens are conveniently studied. The fecal flora, however, is more likely to be representative of the lumenal bacteria, not the bacteria intimately associated with the mucosa. These flora in these two microenvironments differ, since host-derived nutrients such as mucin, and colonic secretions such as secretory antibody and bacteriolytic enzymes, are concentrated in the mucinous layer on top of the intestinal mucosa.

Furthermore, the nutrient levels and enzymatic activities measured in the feces do not represent the conditions prevailing in the right colon, since the nutrients are metabolized and the enzyme activities can be expected to drop as
the fecal matter becomes dehydrated and compacted during transit to the rectum. As the nutrients and enzymatic activities decrease during their transit though the colon, the perturbations of diet modifications will be normalized. Hence, any studies relying on these measurements will underestimate the effects of diet in the right colon (Hill 1981). Similar observations regarding the validity of conclusions based on fecal changes would be expected to pertain to the effects of age, stress, and physicochemical alterations. Alternative approaches such as aspirating the lumenal contents of the intestines at various locations address some of the limitations, yet still assess only the lumenal microenvironment.

Beyond the limitations of fecal analysis, this research is complicated by the sheer number of bacterial species and strains present in the large intestine, as well as by the fact that the composition differs among individual hosts even when genetically identical.

Assorted Factors

Physicochemical conditions affecting the composition of the microflora, the synthesis of bacterial enzymes, and the activity of these enzymes include the pH, reduction potential, and oxygen tension of the intestinal environment. The normal colonic pH is 5.5 to 7.0 (Vince 1986). However, the fermentation of carbohydrates reaching the colon can drive the pH below 5.0. These conditions are well tolerated by the lactic acid bacteria such as Lactobacilli and Streptococci, but other bacteria such as the Enterobacteriaceae, Bacteroides, and Clostridiae do not fare so well. At the other extreme of the pH scale, above pH 8.0, most bacteria do poorly, with the exception of Proteus and some Klebsiellae (Hill 1986).

Beyond decreasing the viability of certain bacteria, or perhaps one mechanism accounting for their decreased viability, pH affects bacterial enzyme
synthesis (Hill 1986). Synthesis of some enzymes, such as amino acid
decarboxylases, are induced only at low pH; deaminases are synthesized under
alkaline conditions only; and cholanoyl 7-dehydroxylase, a bile acid-
metabolizing enzyme, is synthesized only at near-neutral pH.

Oxygen tension clearly affects microflora composition. The intestines are
populated predominantly by anaerobes, to which oxygen is highly toxic. The
stomach and proximal small intestines have enough oxygen present to prevent
the growth of anaerobic bacteria under normal conditions. The expression of bile
acid-metabolizing enzymes is similarly suppressed by oxygen. Finally, bacterial
enzyme activity is also affected by these same parameters.

Intestinal motility is another important determinant of intestinal
microflora activity. In fact, it is touted as the major host defense mechanism
against bacterial overgrowth in the small bowel (Simon and Gorbach 1984).
Without special adaptations to anchor themselves, bacteria will be swept along
with the flow of the intestinal contents and excreted from the body. Under
conditions of abnormally rapid transit, such as diarrhea, the population of
anaerobic bacteria in the large intestine decreases five to six log10 units (Simon
and Gorbach 1984). Under conditions of localized or intestinal stasis, however,
aerotolerant bacteria may reduce the oxygen tension sufficiently to allow
anaerobes to reach ecological niches not usually available to them, such as the
upper reaches of the small intestine.

Furthermore, many bacterial enzymes are produced only at certain stages
of the growth cycle, such as during periods of exponential growth or during the
stationary phase, when growth has come to a standstill (Hill 1986). Since
intestinal motility is an important factor in determining the population density of
the intestinal microflora, it also influences the growth cycle.
Myriads of studies have documented the effect of the diet on the metabolic reactions occurring in the intestines. These range from epidemiological studies demonstrating that high-fat, low-fiber diets are associated with colon and breast cancer (Reddy 1980, Armstrong and Doll 1975), to animal studies showing specific fecal enzyme changes associated with different diets (Simon and Gorbach 1984, Gorbach 1984), to clinical intervention trials confirming the benefits of lactulose for patients with hepatic encephalopathy (Bircher et al. 1971).

Two fundamental mechanisms have been proposed to account for the observed effects of diet, one postulating that the composition of the flora is significantly altered by diet, the other that the activity of the bacterial enzymes is affected. Few studies have found consistent and significant changes in the composition of the fecal flora (Bornside 1978, Hill 1981), even when examining such extreme diets as those of vegans following a "living food" diet of uncooked and fermented foods (Peltonen et al. 1992). In contrast, many studies have documented significant changes in the enzyme activity of fecal bacteria (Reddy et al. 1974, Goldin and Gorbach 1976).

Two physicochemical parameters amenable to modification by the diet have already been discussed. The intestinal pH can be lowered by the presence of undigested carbohydrates reaching the colon. Intestinal motility can be increased by high-fiber diets (Cummings et al. 1979) by a number of mechanisms, including a reflex response to the greater fecal bulk, and more liquid stools due to the increased osmolality of undigested fiber in the stool (Saez 1991). As described above, the effects of altering the physicochemical properties of the intestines can operate in ways which affect enzymatic activities even without significantly altering the composition of the microflora.
Protein-calorie malnutrition is a form of stress. In children it is associated with larger numbers of Streptococci, coliforms, and Bacteroides in the small intestines (Tannock 1983). For changes associated with other types of stress, including emotional stress, see Holdeman et al. (1976).

A study in inbred mice demonstrated that the composition of the intestinal microflora was exceedingly stable in mice with intact immune systems, whereas genetically identical athymic mice showed a highly unstable composition (van der Waaij 1988). The data suggest that the immune system may play a role in excluding exogenous bacteria from colonizing the intestines. Indigenous intestinal flora, when injected parenterally into the host, do not elicit an immune response (Foo and Lee 1972, Berg and Savage 1975). Changes in mucosal immunity associated with aging (Kawanishi 1993) may account for changes observed in the elderly.

The composition of the microflora itself is a strong regulator of its composition. This autoregulation is accomplished by the secretion of antimicrobial substances, competition for sources of nutrition and sites of adhesion to the mucosa, and perhaps by modification of the mucosal immunity.
IV--Probiotics

The human and economic toll of C. difficile-associated disease may be needless. Clinical trials support the findings of microbiologic and animal studies which suggest that there might be medical prophylactic measures available to protect individuals at risk of developing antibiotic- and C. difficile-associated disease. These measures would complement the infection control measures now implemented in many hospitals, which focus on preventing the spread of C. difficile.

Due to the special nature of the disease, the usual prophylactic measures used against bacterial infections cannot be used. Instead of antibiotics, probiotics might be the prophylactic agents of choice. A probiotic is "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance" (Fuller 1991).

The two probiotics most extensively studied as AAD and CDAD prophylactics is a yeast called Saccharomyces boulardii and bacteria of the Lactobacillus genus. Two other probiotic approaches to CDAD include the administration of either fecal enemas or nonpathogenic strains of C. difficile.

Saccharomyces boulardii

First isolated in Indochina from the lychee fruit in the 1920s, S. boulardii was used in France during the 1950s as a treatment for diarrhea. Since then it has become widely used throughout Europe, South America, and Africa (Mcfarland and Bernasconi 1993a). However, only now is it undergoing phase III clinical trials in the United States.
Because it is a yeast, *S. boulardii* has a dramatic advantage over bacterial probiotics, namely that it is unaffected by antibiotic therapy. And unlike many other yeasts, *S. boulardii* grows best at normal body temperature. A third quality vital to its success as a probiotic is its ability to survive transit through the digestive tract (Kimmey et al. 1990).

**AAD**

The potential use of *S. boulardii* as a prophylactic agent against AAD has been investigated in three large double-blind placebo-controlled clinical trials, each involving 180 or more subjects. Two of these studies enrolled hospitalized patients, one studied outpatients; two limited their investigations to one or two antibiotics, while the third considered all antibiotics. All three studies found that *S. boulardii* significantly reduced the incidence of AAD.

The multicenter study of Adam et al. (1977) examined 388 ambulatory patients, aged 15 or older, who were slated to receive at least five days of tetracycline or beta-lactam antibiotic therapy for infectious broncho-pulmonary or otorhinolaryngeal (ENT) diseases. Excluded were "psychologically unreliable" patients and those suffering from diarrhea or disorders that might involve the gastrointestinal tract. Eligible patients were randomly assigned to take either *S. boulardii* or placebo four times a day for the entire duration of their antibiotic therapy. The groups did not differ significantly with respect to age, sex, type and duration of antibiotic and placebo/yeast therapy, principal diagnosis, or past history of complications due to antibiotics and gastrointestinal problems.

The presence and severity of diarrhea reported by patients was established by an index of intestinal disturbance that assigned points for the number and consistency of stools. The total incidence of AAD was four times
higher in the control group (4.5% vs 17.5%, p < 0.001). These patients also suffered significantly more severe diarrhea.

There is no indication of any follow-up after antibiotic and placebo/yeast therapy was discontinued. It would have been valuable to see whether the study group developed AAD after discontinuing the yeast, since it is entirely conceivable that the yeast merely suppressed or delayed the expression of AAD.

Surawicz et al. (1989a) enrolled consecutive inpatients receiving new prescriptions for antibiotics. Exclusion criteria included: diarrhea within the preceding week; immune compromise either as a result of AIDS, chemotherapy, or radiotherapy; renal failure requiring dialysis; pregnancy; medical therapy involving the following medications: vancomycin, metronidazole, antifungal antibiotics, or lactulose. A 2:1 double-blind randomization of S. boulardii to placebo was used. The two cohorts did not differ significantly with respect to gender or mean age, nor did the patients excluded from the study differ from those enrolled. The dose of yeast was 500 mg twice a day. Subjects were initiated into the study within 48 hours of beginning antibiotic treatment, and continued to receive capsules of either yeast or placebo for two weeks after ceasing their antibiotic therapy. Only patients who were monitored for eight or more days were included in the analysis. Diarrhea in this and the other S. boulardii trials discussed below was defined as "a change in bowel habit with three or more loose or watery stools per day for at least 2 days."

Like Adam et al. (1977), Surawicz and associates (1989a) found that S. boulardii administered concurrently with antibiotic therapy could reduce the incidence of AAD (9.5% vs 21.8%, p = 0.038). However, the measured effectiveness of 57% is substantially lower than the 74% reported by Adam and team (1977).
While the *S. boulardii* treatment significantly reduced the incidence of AAD, those among the treatment cohort who ultimately did develop diarrhea suffered a significantly increased mean number of daily bowel movements compared to the placebo group (5.2 vs 3.7, p < 0.05). The test group also showed a longer mean duration of diarrhea and a higher incidence of fever, though these findings were not significantly different from the placebo group. It is conceivable that the yeast itself might be a dose-dependent cause of diarrhea, thereby accounting for the lower effectiveness detected by Surawicz et al. (1989a), who used a larger dose of yeast than Adam et al. (1977). However, subsequent investigations using the same dose did not report similar findings, and there are no documented cases of *S. boulardii*-induced diarrhea in the literature.

An alternate explanation for the decreased effectiveness is the delay of up to two days between the start of antibiotic therapy and the start of the *S. boulardii* prophylaxis. This "window" of vulnerability overlaps with the reported 1-10 day incubation period for *C. difficile*-associated diarrhea after initiating antibiotic therapy (McFarland and Stamm 1986). Some cases of AAD in the test group may thus be attributable to the delay. Consistent with this interpretation is the fact that the incidence 21.8% incidence of AAD in Surawicz et al.'s (1989a) control group is similar to the 17.5% obtained by Adam and associates (1977), while Surawicz et al.'s (1989a) test group experienced a two-fold higher incidence than Adam et al.'s (1977) (9.5% vs 4.5%). The rationale for delaying the administration of the yeast in this and other trials is the belief that the yeast will be more effective in a colon already considerably cleared of *C. difficile* by the antibiotics (Surawicz et al. 1989b).

Another possibility is that *S. boulardii* may delay the onset of AAD, in which case the minimum of 5 days of observation by Adam and associates (1977)
may not have detected all the cases of AAD that would have arisen had the subjects been followed longer. Finally, one must take into consideration that the subjects enrolled by Surawicz and team (1989a) were hospitalized, hence presumably more seriously ill than the ambulatory subjects studied by Adam et al. (1977). A severe underlying illness may conceivably reduce the effectiveness of the yeast.

The third study comes from the same laboratory that conducted the second clinical trial reviewed (McFarland et al. 1993). Instead of enrolling subjects regardless of the antibiotic taken, this study focused on subjects receiving beta-lactam antibiotics such as ampicillin and amoxicillin, either alone or in conjunction with other antibiotics. A 1:1 yeast to placebo randomization was used in this study rather than the 2:1 used in the previous one. A further change in the protocol was the initiation of the yeast or placebo administration at least 72 hours after the antibiotic was begun (vs within 48 hours) and continuing for at least two days after the antibiotic was discontinued (vs two weeks); subjects were then followed for seven weeks for signs of AAD or adverse reactions to the S. boulardii treatment. The same dose of yeast was used.

This trial demonstrated a 56% effectiveness for S. boulardii prophylaxis of AAD, nearly the same as the previous study. The incidence of AAD in both the control and test groups was found to be lower than that of their respective counterparts in the trial by Surawicz and associates (1989a). In contrast to that study, McFarland et al. (1993) report no significant difference in adverse reactions between the yeast and placebo groups.

CDAD

Evidence regarding the ability of S. boulardii to prevent disease specifically caused by C. difficile is considerably scantier, consisting of case reports (Kimmey
et al. 1990), small open trials (Buts et al. 1993, Surawicz et al. 1989b), and one ongoing double-blind placebo-controlled multicenter trial (Surawicz et al. 1993). The clinical trials, both headed by Surawicz, consider *S. boulardii* as an adjunct to conventional antimicrobial therapy. Additional support for the efficacy of *S. boulardii* in preventing CDAD comes from animal studies (Toothaker and Elmer 1984, Elmer and McFarland 1987 and 1990).

The first clinical study was an open trial of 13 patients who, after successful treatment with vancomycin, experienced one or more recurrences of CDAD (range 1-9, mean 3.6 +/- 2.3) and had stools positive for both *C. difficile* and the cytotoxin upon enrolling in the trial (Surawicz et al. 1989b). Capsules of *S. boulardii* (500 mg bid) were given beginning around day 5 of the standard 10-day course of vancomycin therapy and continued for a total of 30 days.

Eleven of the 13 patients (85%) showed resolution of the symptoms of diarrhea or colitis without any further recurrences during the 30 days of *S. boulardii* administration. Seven patients were available for follow-up a median of six days (range 2 - 150 days) after cessation of the *S. boulardii* treatment: none of them reported recurrence of diarrhea.

The authors fail to give their results the modicum of significance available in an open trial by neglecting to compare their finding of a 15% relapse rate within 20 days of vancomycin cessation with an expected rate of relapse had *S. boulardii* not been given. However, the findings of the multicenter placebo-controlled trial initiated by the same laboratory several years later provides some basis for comparison: of 28 subjects with a history of CDAD recurrences enrolled in that trial, 19 (68%) controls suffered relapses during the roughly 50 days of observation after vancomycin or metronidazole treatment was concluded (Surawicz et al. 1993). Since the highest incidence of relapses can be expected to occur shortly after conclusion of antibiotic therapy, it seems unlikely that the
open trial would have found a relapse rate approaching 68% had they continued observation for another month. This would indicate that *S. boulardii* as an adjunct to antibiotic was indeed effective at preventing recurrences of CDAD. At the same time, one must bear in mind that the 15% recurrence rate is based on a very small sample size, and therefore prone to error.

Stools from the 13 patients were positive for both *C. difficile* and cytotoxin as a precondition for their eligibility to be included in the trial. Evaluation of 11 patient’s stools, some during the *S. boulardii* administration but after the course of vancomycin was finished, others after *S. boulardii* treatment finished, demonstrated a significant decrease in *C. difficile* culture and cytotoxin positive stools.

Some of the shortcomings of the open trial are being addressed by the multicenter trial mentioned above (Surawicz et al. 1993). Patients currently experiencing *C. difficile*-associated diarrhea or colitis are receiving *S. boulardii* or placebo for one week during, and for three weeks immediately following, their standard vancomycin or metronidazole treatment. They are then monitored for an additional month after the placebo or treatment is discontinued. Unlike in the open trial, subjects with first time CDAD are eligible in addition to those with recurrences.

Of the 102 patients who have completed the trial so far, 37 (36%) have had recurrences: 26 of these were in the placebo group of 57 (45%), while 11 were in the cohort of 45 subjects receiving *S. boulardii* (24%). The study group has a significantly lower rate of CDAD recurrence (p < 0.05), though the efficacy of the yeast is only 47%.

While the above results support a role for *S. boulardii* in preventing recurrent CDAD, they fail to address its effectiveness against first-time CDAD. Because the pathogenesis of recurrent CDAD has not been clearly established, it
is plausible that a demonstrated effectiveness against one form of CDAD does not necessarily apply to the other. Relevant data on incident CDAD is scant. Surawicz found that 3 (9.4%) of the 32 C. difficile positive patients treated with yeast developed diarrhea, compared with 5 of the 16 (31%) C. difficile positive patients receiving placebo (Surawicz et al. 1989a). This apparent three-fold protective effect of S. boulardii was marginally significant (p = 0.07). McFarland et al. (1993) also found a decrease in the incidence of diarrhea in C. difficile-positive patients taking yeast versus placebo (20% vs 31%), yet this difference likewise failed to achieve statistical significance.

**Mechanism of Action**

*C. difficile*-specific

A study of the effects of *S. boulardii* administered to healthy human volunteers showed no significant changes in the concentration of total anaerobes, *Bacteroides*, or indigenous *Clostridium* (Klein et al. 1993). Yet, as detailed above, *S. boulardii* does protect against CDAD.

One reasonable explanation is that *S. boulardii* exerts its beneficial effects at the level of the toxin instead of by reducing numbers of *C. difficile*. *Saccharomyces boulardii* could have this effect on cytotoxin levels either by reducing toxin production or by inhibiting the cytotoxic effects of the elaborated toxins.

Consistent with this notion, *S. boulardii* treatment of gnotobiotic mice was found to reduce fecal *C. difficile* cytotoxin levels without concurrently reducing the *C. difficile* counts (Corthier et al. 1986). Indeed, cytotoxin levels were reduced two to three log10 units (Castex et al. 1990). This effect is a function of the dose and viability of the yeast (Elmer and Corthier 1991).

The prophylactic effect of *S. boulardii* appears not to be due to a direct action on the toxins or their production. The decreased toxin counts for *C. difficile*
did not result from a loss of pathogenicity, as germ-free mice exposed to the
*S. boulardii*-protected mice all died of PMC (Corthier and Muller 1986). Similarly, *C.
difficile* toxins were not reduced in concentration when incubated with *S. boulardii*
(Corthier et al. 1992):

Instead, the effect seems to be the result of *S. boulardii* interaction with the
host. Scanning electron microscopy of the cecum and small bowel of *S. boulardii*
treated gnotobiotic mice showed almost no morphological changes resulting
from exposure to *C. difficile*, in sharp contrast to the unprotected mice (Castex et
al. 1990). Incubation with *S. boulardii* protected a rat cell line from undergoing
the cytoskeletal changes normally associated with *C. difficile* toxin exposure
(Czerucka et al. 1991).

Recent evidence points to a *S. boulardii*-elaborated protease that degrades
the toxin A receptor (Pothoulakis et al. 1993). *Saccharomyces boulardii*-cultured
medium, whether given as pretreatment or co-administered with toxin A gave
significantly reduced fluid secretion and mannitol permeability in the ileal loops.
Heat-inactivating the medium eliminated this protective behavior. A similar
result was found for the binding of radiolabeled toxin A to mucosal brush
border, insofar as heating abolished the ability of *S. boulardii* to prevent binding.
Preincubation of *S. boulardii* conditioned medium with a protease inhibitor
likewise abolished the protective effect. Sodium dodecyl sulfate polyacrylamide
gel electrophoresis (SDS PAGE) of purified rat ileal brush borders exposed to *S.
boulardii*-conditioned medium that had been filtered showed a distinct pattern
consistent with protein degradation when compared to medium alone or *S.
boulardii*-conditioned medium that had been heat-inactivated or preincubated
with protease inhibitors.

The preceding presentation gives a coherent picture of the mechanism
involved. Unfortunately, things may not be so tidy. The same laboratory that
reported a decrease in toxin titers without a concomitant loss of \textit{C. difficile} counts or pathogenicity (Corthier and Muller 1986) announced two years later that gnotobiotic mice protected from \textit{C. difficile} infection with \textit{S. boulardii} did develop significant levels of nontoxigenic \textit{C. difficile} in their stools (Corthier et al. 1988). These nontoxigenic \textit{C. difficile} were shown to derive from the original challenging clone, and despite being mutants, within 30 days became as numerous as their toxigenic counterparts. It is proposed that these nontoxigenic clones have an antagonistic effect upon toxigenic strains. The possible implications of this finding are discussed at greater length below.

Non \textit{C. difficile}-specific

The role of \textit{Candida albicans} in diarrhea has long been debated. In immunocompromised animal models and human beings, this yeast has been shown to translocate out of the intestines to become disseminated to other parts of the body. Orally administered \textit{S. boulardii} significantly reduces the incidence and number of \textit{C. albicans} organisms translocating to the mesenteric lymph nodes, spleen, liver, and kidneys in mice (Berg and Savage 1993). Translocation of \textit{C. albicans} across the mucosa following intestinal overgrowth is the dominant mechanism proposed for systemic candidiasis (Stone et al. 1974).

The effect of cholera toxin can be neutralized by \textit{S. boulardii}-conditioned medium (Czerucka et al. 1994). This effect, in contrast to the neutralization of \textit{C. difficile} toxin, is not associated with proteolytic activity. Instead, it seems that \textit{S. boulardii} secretes a protein that binds to a receptor that is negatively coupled to adenylate cyclase, perhaps the pertussis receptor itself.

Both human and rat small intestinal mucosa showed a marked increase in the activity of mucosal disaccharidases after 15 days of oral \textit{S. boulardii} treatment.
(Buts et al. 1986). This may be a significant finding, since diarrhea can be caused by the increased osmotic pressure of undigested carbohydrates in the intestines.

Oral administration of *S. boulardii* for seven days was demonstrated to activate the reticuloendothelial and complement system in healthy human volunteers (Caetano et al. 1986). Another study found that secretory component and secretory IgA are significantly elevated in rats treated with *S. boulardii* (Buts et al. 1990). Both of these findings argue for a general salutatory effect of *S. boulardii* ingestion.

**Lactobacilli**

The medical notion of using lactobacilli as probiotics predated the introduction of the term "probiotic" by nearly seventy years. In 1908, Mechnikov observed that centenarians abounded in Bulgaria (Fuller 1991). He attributed their longevity to the prominent role of yogurt in their diet and reasoned that the acid-producing bacteria could be used for treating illnesses arising from the colon. Since then, many intervention studies have purported to find beneficial effects of lactobacillus preparations on a wide variety of ailments.

Recent investigations have focused mainly on one of two lactobacillus preparations. Lactinex is a commercially available mixture of viable *L. acidophilus* and *L. bulgaricus* supplemented with whey powder, dried milk, talc, and lactose. *Lactobacillus GG* is a patented strain, selected for its ability to adhere tightly to intestinal mucosa, colonize human intestines, and elaborate a potent antimicrobial substance (Gorbach 1990).

**AAD**

Four clinical trials have tested the effectiveness of a lactobacillus preparation as a prophylactic agent against AAD. The trials, published between
1979 and 1990, each examine a different commonly used antibiotic. Three of them studied the effectiveness of Lactinex, while the fourth used *Lactobacillus* GG. All the published studies enrolled fewer than 100 subjects, and two had less than 40.

Three of the studies are randomized placebo control studies; two of these are double-blind. Two studies were conducted on healthy volunteers challenged with bacteria known to cause diarrhea. The definition of diarrhea differed in all four studies. Three of the studies found a prophylactic effect. The one study that failed to find any effect was conducted on children, while the subjects in the three others were adults.

In the first double-blind placebo-controlled study, Gotz et al. (1979) enrolled ninety-eight hospital inpatients screened within 24 hours of the initiation of ampicillin therapy. The screening excluded patients who had underlying conditions with diarrhea as a constitutional factor, patients receiving multiple antibiotics, or those not eating a normal diet. Enrolled subjects were randomly assigned to receive either Lactinex granules or a placebo four times daily for the first five days of ampicillin therapy. The placebo, supplied by the manufacturer of Lactinex, consisted of the substrate of the Lactinex packets without the viable bacteria. Seventy-nine (81%) patients completed the study. While no analysis is given of the 19 patients who did not complete the study, the placebo and Lactinex groups are reported not to have differed with respect to age, dose of ampicillin, or the number of doses of the study drug.

Subjects were monitored for diarrhea throughout the duration of ampicillin therapy. Diarrhea was defined as three or more bowel movements beyond the subject's normal daily routine as established during the initial interview. Before the double-blinding code was broken, the three investigators independently reviewed each case of diarrhea, categorizing it with respect to
etiology as either ampicillin-associated or ampicillin-unrelated; the former category was then further subdivided.

The study found a higher attack rate of diarrhea of unspecified etiology among the recipients of the placebo, but the difference did not achieve statistical significance (p = 0.21, Fisher's Exact Test). When the cases of diarrhea were categorized according to etiology, however, among the cases of diarrhea attributed to ampicillin, the greater attack rate for the placebo cohort achieved statistical significance (p = 0.03) with Fisher's Exact Test. Gotz and associates (1979) concluded that Lactinex may be effective at preventing ampicillin-associated diarrhea in adults. To consolidate their findings the group demonstrated in a follow-up randomized three-way cross-over study that Lactinex does not interfere with oral ampicillin bioavailability (Yost and Gotz 1985).

Tankanow et al. (1990) conducted a double-blind, placebo-controlled study of children taking amoxicillin. Excluded from the study were children currently experiencing diarrhea or an underlying condition with diarrhea as a typical symptom; were not eating a normal diet prior to developing their current illness; were being nourished by mothers' milk only; had known sensitivities or contraindications to Lactinex, lactose, or milk products; were receiving drugs known to interact with amoxicillin. Sixty eligible children from a local pediatric practice were randomly assigned either Lactinex or a placebo to be taken four times a day for 10 days while concurrently undergoing amoxicillin therapy on an outpatient basis. Thirty-eight (63%) children completed a minimum of five days of antibiotic and Lactinex/placebo therapy, and were consequently evaluated by the investigators. Their ages ranged from between five months to six years.

Diarrhea in this study was defined as one or more abnormally loose bowel movements per day. More than two-thirds of the subjects completing the study
experienced diarrhea, but the attack rate was not found to differ between the study and control groups. Likewise, the incidence of diarrhea diminished during the last four days of therapy for the Lactinex subjects, while it remained constant for the placebo group. No indication is given as to whether this observation was statistically significant. Chi-square analysis indicated that the rate of diarrhea among children under three years of age was not significantly higher than that among children three to six years of age.

A major problem interpreting the work was the high loss of study subjects. Tankanow and associates (1990) initially found 100 eligible subjects, of whom 60 agreed to participate, though only 38 completed the minimum of five days of combined amoxicillin and Lactinex/placebo therapy. No information is provided on the profile of those subjects who withdrew from the study. Likewise, there was no comparison of the relative composition of the study and control groups with respect to age, gender, and severity of the diagnosed illness for which amoxicillin was prescribed.

Tankanow et al. (1990) cite Kramer and associates (1985) as having demonstrated that “the pediatric population may be the most susceptible to diarrheal episodes following antibiotic therapy.” At the same time, there is extensive documentation that children are far less susceptible to CDAD than adults (McFarland and Stamm 1986), even when they carry high levels of C. difficile toxins in their stool (Lyerly et al. 1988). Thus, assuming that the composition of the control and study groups were representative of the original target population, the study at best demonstrated that Lactinex has no effect at prophylaxing against “enigmatic” AAD.

The third trial of Lactinex’s effectiveness involved a total of 48 healthy volunteers aged 18 to 35 (Clements et al. 1983). Diarrhea here was cumbersomely defined as three or more unformed stools, or two unformed stools totaling at
least 200 ml, or a single liquid stool >300 ml. The volunteers were randomly assigned to receive either Lactinex or placebo four times a day beginning three hours after the first dose of neomycin and continuing throughout the five-day period of neomycin treatment. Two different batches of Lactinex were examined serially.

The volume and number of stools experienced by volunteers who received the first batch of Lactinex were both significantly smaller (p = 0.001 and p = 0.05, respectively) than that experienced by the placebo group. The attack rate and duration of diarrhea were also reduced, but not significantly. The second batch of Lactinex failed to provide any significant difference. Subsequent analysis of the two batches failed to provide any explanation for the observed discrepancy, having similar counts of viable *L. acidophilus* with similar fermentation patterns and colony types. This lot-to-lot variation may explain some of the lack of reproducibility and inconsistencies that have plagued research of lactobacilli’s probiotic potential.

Siitonen and team (1990) examined the ability of *Lactobacillus* GG to protect healthy, young, male volunteers against erythromycin-associated diarrhea and other gastrointestinal symptoms. The 16 subjects were randomly assigned to receive yoghurt that was either pasteurized or contained live *Lactobacillus* GG cultures. Blood and stool samples from both groups were taken on the first and last day of the seven-day trial to assess drug levels and lactobacilli levels, respectively.

The reported incidence of self-assessed diarrhea was significantly lower (p < 0.05) in the GG yoghurt group, though no data were provided. The more objective measure of diarrhea, fecal volume, was measured on the first and last day and were not different for the two groups. Nonetheless, the authors concluded that *Lactobacillus* GG did significantly reduce the incidence of
diarrhea. There is no indication of whether the subjects could detect the difference between a pasteurized yoghurt and one fermented with *Lactobacillus GG*.

**CDAD**

None of the lactobacilli trials above identified culture- or toxin-positive *C. difficile* cases. Thus, what prophylactic effects of lactobacillus preparations were found cannot be reliably extended to the prevention of *C. difficile*-associated diarrhea, which comprises only 15-25% of all AAD. Furthermore, because the association of *C. difficile* with antibiotic therapy becomes stronger as the severity of the antibiotic-induced condition increases, the less stringent definitions of diarrhea used in the above studies probably minimized the detection of a lactobacillus effect on *C. difficile*.

There is, however, weak evidence that these lactobacillus preparations may prevent relapses of CDAD. Much of the evidence is anecdotal, but some comes from an open trial. Gorbach, one of the patent-holders of *Lactobacillus GG*, has been conducting an on-going trial with patients who suffer from multiple recurrences of *C. difficile* colitis (Gorbach 1990). As of 1990, 11 patients had been treated with *Lactobacillus GG*. Eight of these experienced immediate resolution of their symptoms, no further recurrences, and negative or very low titers of toxin in their stools. Three patients relapsed again after one treatment of the probiotic, but did not have a subsequent recurrence after a second treatment (Gorbach 1990). Some of these patients have been followed for over four years (Gorbach et al. 1987).

Young and associates (1985) reported on seven patients with a mean of 2.6 symptomatic relapses of CDAD following standard vancomycin or bacitracin therapy. Disease in these patients remitted completely when treated with yet
another course of vancomycin followed by 1-2 weeks of eating yoghurt containing viable lactobacilli.

Bennet et al. (1990) administered *Lactobacillus* GG for 7-14 days to nine nursing home patients with symptomatic recurrences of CDAD. For 60 days they all showed improvements and did not require any antibiotic therapy, but 4 (44%) had recurrences after 60-180 days. While this rate of recurrence is similar to that reported in the literature, the recurrences are normally seen far sooner than 60 days after treatment, suggesting that these recurrences actually represent cases of reinfection.

**Mechanism of Action**

Ironically, despite a much longer history of medical claims, lactobacilli remain less clearly understood than *S. boulardii*, with respect to mechanism of action. There are several plausible reasons for this, including historical, economic, and microbiologic.

*Saccharomyces boulardii* was supplied by a single manufacturer in virtually all of the research cited. This assures a modicum of consistency in the preparation, character, and viability of the product. If strains and subspecies of the yeast exist, they can be excluded from the preparations.

The lactobacilli studied in the literature, on the other hand, derived from a multitude of sources. Such variability, compounded by the taxonomic complexities of the genus, can result in the study of different strains of the same species (Bottazzi 1988). In fact, the *Lactobacillus* literature covers different species such as *L. acidophilus*, *L. bulgaricus*, and *L. casei*, sometimes individually, other times in combinations. Even when supplied by the same manufacturer, the activity is apparently not uniform across different batches, as seen in the study of Clements and associates (1983) experience with Lactinex.
Compounding this confusion, there have been no clinical trials of lactobacilli that match those of *S. boulardii*, either with regards to size or scientific rigorousness. The net effect is a haphazard collection of conflicting publications with insufficient power to draw meaningful conclusions. Issues of bias aside, the reason for such a discrepancy may ultimately be due to the lack of corporate financing for clinical research of *Lactobacillus* products: the clinical trials of *S. boulardii* are virtually all funded by the marketer of the yeast, Biocodex, Inc.

Another explanation, more forgiving of the field, may be due to difficulties associated with the mechanisms whereby lactobacilli act. It is understandably easier to identify and characterize a filtratable product than it is to cleanly establish the basis for a phenomenon such as colonization resistance, which by its very nature defies abstraction to simplified laboratory techniques.

Despite 15 years of literature touting the ability of lactobacilli to prevent AAD, and seven years of results suggesting that lactobacilli can control recurrent PMC, very little has been published concerning the possible mechanisms for such actions.

Indeed, very few publications even explore the interactions between lactobacilli and *C. difficile*. Lactinex prevented the development of ileocecalis in hamsters treated with antibiotics and challenged with *C. difficile* (Winans et al. 1980). Lactobacilli were shown to inhibit the multiplication of *C. difficile in vitro* (Rolfe et al. 1981). The toxin B can be inactivated by the myeloperoxidase system of neutrophils given a source of hydrogen peroxide generated by glucose oxidase or lactobacilli (Ooi et al. 1984). *Lactobacillus* GG produces a substance of low molecular weight that is active against a wide range of bacteria, including *Clostridium* spp. (Silva et al. 1987). Amazingly, this represents the extent of the literature.
Lactobacilli elaborate a variety of antibacterial compounds. Bacteriocins are proteinaceous bactericidal compounds with a narrow inhibitory spectrum. Most are active against species closely related to that of the producer, though the bacteriocins of Gram-positive bacteria have a somewhat broader spectrum activity (Havenaar et al. 1992). Antimicrobials such as acidolin, acidophilin, and bulgarican have activity against both Gram-positive and Gram-negative. More recently, an antimicrobial dubbed reuterin after the species *L. reuteri* covers not only all bacteria tested but yeasts, fungi, and even a protozoan (Havenaar et al. 1992).

Adherence has been demonstrated for several species of lactobacilli, but this property varies considerably: strains of a given *Lactobacillus* species may adhere to different host animal species. Claims of colonization of the human intestinal tract have been made for *Lactobacillus GG* (Ling et al. 1992, Millar et al. 1993). In trials demonstrating *Lactobacillus GG* colonization select fecal enzyme activities were significantly reduced (Ling et al. 1992), but in preterm infants there was no evidence of any positive clinical benefit (Millar et al. 1993).

Various attempts have been made to demonstrate the ability of lactobacilli to prevent Traveller's diarrhea, a disorder most frequently caused by enterotoxigenic *E. coli* (ETEC). Many attempts have failed, though some have succeeded (Oksanen et al. 1990, Lidbeck and Nord 1993). Experiments *in vivo* have demonstrated that Lactinex significantly reduced the fluid response of rabbit ileal loops to preformed ETEC toxins (Johnson et al. 1979, Foster et al. 1980). Though the mechanism for this effect was not specifically addressed, the authors suggest that the species of *Lactobacillus* in Lactinex were competing with the toxins for binding sites (Foster et al. 1980). The relevance of this literature to the issue of CDAD prevention is further reduced by the fact that ETEC toxins
predominantly affect the small intestine (Ryan 1990), while C. difficile toxins affect
the large intestine.

Indirect mechanistic evidence in support of a role for lactobacilli in the
prophylaxis against CDAD can be gleaned from the substantial body of literature
that exists supporting the other health claims made by the lactic acid bacteria
lobby. An immune-stimulating role has also been proposed for Lactobacillus GG.
Children with acute diarrhea recover faster when given this probiotic (Isolauri et
al. 1991). Lactobacillus GG administered to children experiencing acute rotavirus
diarrhea significantly reduced the duration of diarrhea (Kaila et al. 1992). This
improvement was associated with a significantly enhanced nonspecific humoral
response that included IgA, IgM, and IgG. A specific IgA response to rotavirus
was also present in significantly more children treated with the Lactobacillus.

Many other health claims have been made for lactobacillus preparations.
These include such nutritional benefits: increased bioavailability of metals
(McDonough et al. 1983); increased levels of riboflavin, niacin (Alm 1982),
pantothenic acid (Deeth and Tamine 1981), and folic acid (Shahani and Chandan
1979); higher levels of free amino acids due to bacterial proteolysis (Alm 1982).
Therapeutic benefits include treatment of vaginitis (Klebanoff et al. 1991),
anticarcinogenic, antihypercholesterolemic, and lactase deficiency-alleviating
effects (Goldin and Gorbach 1992).

**Fecal Enemas**

This unpleasant-sounding technique represents the logical conclusion of
research on colonization resistance. Investigators have been unable to identify a
subset of the intestinal microflora capable of fully reconstituting colonization
resistance in gnotobiotic animals and animals whose intestinal flora has been
radically altered by antimicrobials (Wilson et al. 1988b). Instilling preparations
of normal feces into these animals, however, fully reconstitutes their resistance to intestinal pathogens. In fact, this technique has long been used in animal husbandry (Fuller 1991).

No double-blind placebo-controlled trials have been conducted using fecal enemas. Yet the literature is peppered with case reports suggesting that these enemas were effective at treating in 13 of 16 patients with PMC (Bowden et al. 1981) and preventing its recurrence in one patient (Schwan et al. 1981). Anecdotal evidence of serious enteric disease transmitted by such enemas attest to the perils of this treatment modality. A safer approach was demonstrated effective by Tvede et al. (1990), who used a suspension of 10 different species of aerobes and anaerobes isolated from feces (Tvede and Rask-Maden 1989).

**Nonpathogenic *C. difficile***

Ample evidence exists that hamsters can be protected from *C. difficile* ileocecalitis by prior colonization with nonpathogenic strains of *C. difficile* (Wilson and Sheagren 1983, Borriello and Barclay 1985, Corthier et al. 1988). Seal et al. (1987) extended these findings to human beings by successfully treating two patients with relapsing colitis by administering a nontoxigenic isolate of *C. difficile*.

Nontoxigenic strains of *C. difficile* have been shown to emerge in gnotobiotic mice challenged with toxigenic *C. difficile* after being treated with *S. boulardii* (Corthier et al. 1988) or special diets (Mahe et al. 1987, Corthier et al. 1988). These nontoxigenic strains of *C. difficile* were shown to derive from the original challenging clone (Corthier et al. 1988).

Nontoxigenic clones seem to have two distinct protective properties: colonization prevention and toxin suppression. In order to prevent colonization by toxigenic strains, germ-free mice must be fed nontoxigenic strains at least 18
hours before receiving a challenge with a toxigenic strain (Corthier et al. 1988). Giving the toxigenic strain sooner than 18 hours failed to demonstrate this protection, suggesting the need for the nontoxigenic strain to become established in order to protect against the toxigenic clone. Wilson and Sheagren (1983) reported similar findings with *C. difficile*, and antagonism between isogenic strains was demonstrated in *E. coli* (Duval-Iflah et al. 1981).

To suppress toxin levels, there does not seem to be a need for the nontoxigenic strain to be established. Nontoxigenic strains that arose from a toxigenic clone during *S. bourlandii* administration or diet manipulation competed successfully with their toxigenic counterparts, eventually reaching similar titers. At the same time, the levels of cytotoxin were inversely proportional to the titers of nontoxigenic bacteria (Corthier et al. 1988).

These clones are stable: 10 successive subcultures did not cause reversion to toxigenicity. Moreover, several mutations may be involved, as some clones exhibited intermediate levels of cytotoxin production (Corthier et al. 1988). Neither cytotoxin nor enterotoxin, nor factors affecting the expression of these toxins, have been found to be coded on plasmids or bacteriophage.

**Relative Merits of Each Strategy**

To assess the relative merits of each of the proposed probiotic strategies to control CDAD, one must first establish what are the desired goals of the intervention, be they prevention of initial or recurrent disease, palliation of acute disease, or effective decontamination of the hospital environment. While these goals are not mutually exclusive, the probiotics differ sufficiently in their strengths to warrant separate consideration.

*Saccharomyces bourlandii*, the probiotic boasting the greatest CDAD-specific literature, appears able to decrease the incidence of symptomatic disease,
whether incident or recurrent, by interfering with the action of the toxins. In addition, being a yeast, the probiotic is not likely to be directly affected by concurrently administered antibiotics.

While these features may be sufficient for most clinicians, hospital infection control personnel should not be so fast to embrace a probiotic with its qualities. Asymptomatic excretors of *C. difficile* are a significant source of environmental contamination, and any intervention that further camouflages such excretors will only exacerbate the problems experienced by hospitals in their attempts to control the spread of this pathogen. Since it hardly seems feasible to prophylax the entire hospital population upon entry, and since there will always be treatment failures, hospitals must continue to consider environmental contamination regardless of how effective a prophylactic is.

Very little is known about lactobacilli with respect to CDAD. Nevertheless, the potential that *Lactobacillus* prophylaxis could be effective by maintaining colonization resistance makes it an attractive candidate for consideration by hospitals. If fewer people acquired the bacterium while in the hospital, *ceteris paribus* there would be a concomitant decrease in its spread. In addition, claims that it nonspecifically stimulates the immune system make it a potentially attractive, relatively inexpensive and well-tolerated strategy deserving of consideration.

Fecal enemas may well be the most effective probiotic method of treating recurrent CDAD. However, given the relative invasiveness, the assault on esthetic sensibilities, and the potential dangers of infusing fecal material and its possible contaminants into a person whose disease resistance is already compromised, this method is has limited prospects. It should probably be restricted to intervention in cases of protracted disease; either suspensions of known isolates or fecal material obtained from intimate associates, who may be
expected to already share similar fecal contaminants such as enteroviruses and parasites, should be used.

By far the most attractive of the proposed probiotic approaches is the use of nontoxigenic strains of *C. difficile*. Although research in this area is admittedly the scantiest, it is conceptually the most promising. Such strains appear to be stable, obviating the most troublesome prospect, that of reversion. People who are at risk of acquiring *C. difficile* in the hospital will benefit from the treatment, and will continue to benefit as long as they continue be vulnerable to the pathogen; people whose colonization resistance is intact will likewise not be affected by the probiotic doses. From the vantage of infection control, contamination of the environment with this strain, whether as a result of patient excretion or deliberate seeding, would only help to further reduce colonization with toxigenic strains of *C. difficile*. 
V--Conclusion

This review has sought to present a critical evaluation of what the literature can tell us about *C. difficile* and its associated disease that will help to formulate an alternate yet rational approach to the treatment and prophylaxis of CDAD. The high and possibly increasing incidence of CDAD among hospitalized patients, the great cost incurred in treating them, and the significant morbidity and mortality they experience are all features of the disease that call for an improved therapeutic approach.

Three states of *C. difficile* infection are identifiable, each perhaps with its own associated risk factors: the act of acquiring the pathogen in the first place, its asymptomatic but stable colonization of the colon, and disease caused by the pathogen. The role of acquisition and carriage in progression to frank disease is a vitally important issue left unresolved by the scientific literature. Certainly, people must become infected in order to develop disease, but there are suggestions that the vulnerability to disease is greatest shortly after infection, a situation seen with tuberculosis albeit on a much shorter time scale. Asymptomatic carriage is clearly important insofar as it results in clinically undetectable environmental contamination.

Environmental contamination is a crucial determinant of the epidemiology of CDAD. Epidemiological evidence supports the notion that *C. difficile* infection is contracted primarily in hospitals and LTCFs. Recovery of infectious *C. difficile* spores in these institutions is typically high. The heavy environmental contamination may well be a prime reason that *C. difficile* is a nosocomial pathogen. Although other host factors have been clearly identified as instrumental in developing CDAD, the fact that hospital in-patients and out-
patients are increasingly similar with respect to these factors would lead one to expect to see a parallel increase of out-patient CDAD if exposure to the hospital environment were not a crucial determinant of disease. No such trend has been reported.

The literature has established that antibiotic use is probably an important risk factor for acquiring and carrying *C. difficile* and certainly important for developing disease. Strong evidence supports the conclusion that age and the severity of underlying non-CDAD illness are significant risk factors for carriage; age is also significantly associated with CDAD. Other host factors, including humoral immune competence, length of hospital stay, recent acquisition of *C. difficile* infection, certain medical procedures and non-antimicrobial medications, have been implicated but are still controversial.

Public health and medical efforts that address the features of hospitals and their patients that promote *C. difficile* acquisition and disease need to be promulgated. Measures to control nosocomial *C. difficile* infection have been instituted in many hospitals throughout the country. These measures are variably successful at controlling outbreaks of nosocomial CDAD: the use of vinyl gloves (Johnson et al. 1990b), disposable thermometers (Brooks et al. 1992), or hospital-wide restriction of clindamycin use (Pear et al. 1994) have been associated with decreases in the number of new cases. Such measures may also be effective in reducing the incidence of endemic cases (Swartzberg, personal communication).

Despite the fact that such measures may reduce hospital-wide incidences of CDAD, they still do not fully protect the patients at risk. To introduce efficient population-specific prophylactic measures it is important to identify these patients. The epidemiological studies reviewed above contribute a sketchy profile of the population at risk. But much more still needs to be established.
One blatant area of ignorance is the patient with a history of CDAD: it is clear that the risk of CDAD in such a person decreases with time, insofar as recurrences usually arise within 60 days. However, what is not clear is whether such patients retain a greater risk of CDAD throughout their lives or whether they quickly return to baseline.

Prophylaxis is usually achieved with antibiotic treatment. In the case of CDAD, however, such measures are not helpful and may indeed prove counterproductive. This is where probiotic prophylaxis becomes desirable. Although human cultures throughout the world have unwittingly been practicing probiotic prophylaxis against intestinal disorders through the ingestion of fermented foods, the medical literature on probiotics use is extremely limited.

Four probiotics were examined in this review, three of which are candidates for prophylaxis against CDAD: even if the dangers of introducing a pathogenic fecal contaminant could be obviated by the development of a mixture of well-characterized bacterial isolates that could reproduce the effectiveness of fecal enemas, the procedure is too invasive for prophylactic use. As covered in Section IV, the remaining three agents each have their own strengths and weaknesses as prophylactic agents. Much more study is needed on lactobacillus and nontoxigenic C. difficile probiotics, though ultimately these two approaches could prove far more effective than S. boulardii insofar as they may be able to prevent infection as well as disease.

Although probiotics have been used extensively for promoting and maintaining health for thousands of years, and their potential as adjuncts to medical treatment has been proposed for nearly one-hundred years, it is only in the past decade that rigorous clinical research on the topic was begun in the United States. Similarly, it is only within the past decade that the notion of using
natural predatory species of insects as an alternative to chemical pesticides to control pests has achieved prominence. What binds these two seemingly disparate areas of human endeavor is the concept of working with the environment rather than against it, of realizing that in complex ecological systems simple solutions do not exist. A new academic discipline has developed that can shed understanding on the interaction between antibiotics and the intestinal microflora just as readily as it can on the interaction between pesticides and the agricultural flora: the study of chaos (non-linear dynamical systems) and complex systems. Hopefully, as this fledgling science matures it will provide great insights into the host-parasite relationship and contribute to a new model of disease causation.
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