

## Shea Position Paper

# CLOSTRIDIUM DIFFICILE-ASSOCIATED DIARRHEA AND COLITIS

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### ABSTRACT

**OBJECTIVES:** To review and summarize the status of diagnosis, epidemiology, infection control, and treatment of *Clostridium difficile*-associated disease (CDAD).

**DIAGNOSIS:** A case definition of CDAD should include the presence of symptoms (usually diarrhea) and at least one of the following positive tests: endoscopy revealing pseudomembranes, stool cytotoxicity test for toxin B, stool enzyme immunoassay for toxin A or B, or stool culture for *C. difficile* (preferably with confirmation of organism toxicity if a direct stool toxin test is negative or not done). Testing of asymptomatic patients, including those who are asymptomatic after treatment, is not recommended other than for epidemiologic purposes. Lower gastrointestinal endoscopy is the only diagnostic test for pseudomembranous colitis, but it is expensive, invasive, and insensitive (51% to 55%) for the diagnosis of CDAD. Stool culture is the most sensitive laboratory test currently in clinical use, but it is not as specific as the cell cytotoxicity assay.

**EPIDEMIOLOGY:** *C. difficile* is the most frequently identified cause of nosocomial diarrhea. The majority of *C. difficile* infections are acquired nosocomially, and most patients remain asymptomatic following acquisition. Antimicrobial exposure is the greatest risk factor for patients, especially clindamycin, cephalosporins, and penicillins, although virtually every antimicrobial has been implicated. Cases of CDAD unassociated with prior antimicrobial or antineoplastic use are very rare. Hands of personnel, as well as a variety of environmental sites within institutions, have been found to be contaminated with *C. difficile*, which can persist as spores for many months. Contaminated com-

modes, bathing tubs, and electronic thermometers have been implicated as sources of *C. difficile*. Symptomatic and asymptomatic infected patients are the major reservoirs and sources for environmental contamination. Both genotypic and phenotypic typing systems for *C. difficile* are available and have enhanced epidemiologic investigation greatly.

**INFECTION CONTROL:** Successful infection control measures designed to prevent horizontal transmission include the use of gloves in handling body substances and replacement of electronic thermometers with disposable devices. Isolation, cohorting, handwashing, environmental disinfection, and treatment of asymptomatic carriers are recommended practices for which convincing data of efficacy are not available. The most successful control measure directed at reduction in symptomatic disease has been antimicrobial restriction.

**TREATMENT:** Treatment of symptomatic (but not asymptomatic) patients with metronidazole or vancomycin for 10 days is effective; metronidazole may be preferred to reduce risk of vancomycin resistance among other organisms in hospitals. Recurrence of symptoms occurs in 7% to 20% of patients and is due to both relapse and reinfection. Over 90% of first recurrences can be treated successfully in the same manner as initial cases. Combination treatment with vancomycin plus rifampin or the addition orally of the yeast *Saccharomyces boulardii* to vancomycin or metronidazole treatment has been shown to prevent subsequent diarrhea in patients with recurrent disease [*Infect Control Hosp Epidemiol* 1995;16:459-477].

### INTRODUCTION

*Clostridium difficile* is a spore-forming gram-positive anaerobic bacillus that produces at least two exotoxins: toxin A, primarily an enterotoxin, and toxin

B, a cytotoxin. The organism causes gastrointestinal infections in humans that range in severity from asymptomatic colonization to severe diarrhea, pseudomembranous colitis (PMC), toxic megacolon,

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TABLE 1  
SENSITIVITY AND SPECIFICITY OF TESTS FOR THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE \*

| Test                                      | Sensitivity  | Specificity  | Utility of Test   | References           |
|---|--------------|--------------|---|----------------------|
| Endoscopy                                 | 51%          | -100%        | Diagnostic of PMC   | 14,16                |
| Culture for <i>C difficile</i>            | 89%-100%     | 84%-99%      | Highly sensitive; confirmation of organism toxicity optimal | 12-14,31,33,49       |
| Cell culture cytotoxin test               | 67%-100%     | 85%-100%     | With clinical data, is diagnostic of CDAD                   | 12-14,31-33          |
| EIA toxin test                            | 63%-99%      | 75%-100%     | With clinical data, is diagnostic of CDAD                   | 11,12,28,32,33,35-42 |
| Latex test for <i>C difficile</i> antigen | 58%-92%      | 80%-96%      | Less sensitive and specific than other tests; rapid results | 11,12,31,38          |
| PCR toxin gene detection                  | Undetermined | Undetermined | Research test   | 61-64                |

Abbreviations: PMC, pseudomembranous colitis; CDAD, *Clostridium difficile*-associated disease; EIA, enzyme immunoassay; PCR, polymerase chain reaction.

\* Using both clinical and test-based criteria.

colonic perforation, and death.<sup>1-3</sup> Two studies have shown *C difficile* to be the most frequently identified cause of nosocomial diarrhea.<sup>4,5</sup> Multiple carefully performed studies have demonstrated the nosocomial acquisition of *C difficile*, both symptomatic and asymptomatic, and the contamination of the hospital environment and the hands of hospital personnel.<sup>6-8</sup>

Despite the large amount of data regarding the etiology of *C difficile* disease and its importance as a nosocomial pathogen, there remain major questions regarding diagnosis, epidemiology, infection control measures, and treatment of this increasingly frequent hospital infection problem. It is the purpose of this position paper to review and summarize the present state of information on *C difficile* disease in each of these four areas: diagnosis, epidemiology, infection control, and treatment. In discussing controversial areas, prevailing and alternate positions have been included. One of the more controversial areas, diagnosis, has been emphasized because case definition and diagnosis are fundamental to any discussion of epidemiology, treatment, and control.

## DIAGNOSIS

For clarity, we define patients as having *C difficile*-associated disease (CDAD) if they display symptomatic illness caused by *C difficile*. Generally, it is agreed that patients in whom a diagnosis is to be made should be symptomatic, and, for the vast majority of patients, diarrhea is the most prominent symptom.<sup>9-11</sup> Whereas asymptomatic *C difficile* gastrointestinal carriage is common in hospitalized patients (and it may be useful from an epidemiologic viewpoint to identify these patients), there is, at this time, no demonstrable clinical benefit to the patient to have the diagnosis of asymptomatic carriage made. Detection of the presence of a *C difficile* toxin in the stool of patients with diarrhea has been the most generally

accepted method of diagnosis; but the sensitivity of stool toxin assays has been questioned, and stool culture has been advocated as a more sensitive, albeit not as specific, alternative.<sup>9-11</sup> Because of these unsettled issues and the fundamental importance of diagnosis, the various diagnostic modalities are presented in detail, and their uses are summarized in Table 1.

## Definition of CDAD

The diagnosis of CDAD should be based on clinical, as well as laboratory, findings. A case definition for the usual presentation of CDAD includes the presence of (1) diarrhea, defined by a variety of criteria (eg, at least six watery stools over 36 hours,<sup>12</sup> three unformed stools in 24 hours for 2 days,<sup>6</sup> or eight unformed stools over 48 hours<sup>13</sup>); (2) pseudomembranes seen at lower gastrointestinal endoscopy, or toxin (A or B) detected in the stool, or a stool culture positive for the presence of a toxin-producing *C difficile*; and (3) no other recognized etiology for diarrhea (rarely, CDAD may coexist with other causes of diarrhea, eg, in AIDS or inflammatory bowel disease). A history of treatment with antimicrobial or antineoplastic agents within the previous 8 weeks is present in virtually all patients,<sup>14</sup> but is not included in the case definition to avoid bias and to allow comparison of antimicrobial use as a risk factor. In clinical practice, antimicrobial use is considered to be part of the operative CDAD definition. A response to specific therapy for CDAD is suggestive of the diagnosis and may be viewed as confirmatory evidence. Rarely (<1%), a symptomatic patient will present with ileus without prior diarrhea. Diagnosis in these patients is difficult; the only specimen available may be a small amount of formed stool or a swab of stool obtained either from the rectum or from within the colon via endoscopy. In such cases, it is important to communicate to the laboratory the necessity to do a toxin assay

or culture for *C difficile* on the nondiarrheal stool specimen.

### **Diagnostic Techniques**

Accurate diagnosis is crucial to the overall management of this nosocomial infection. Empiric therapy without diagnostic testing is inappropriate if diagnostic tests are available, because, even in an epidemic environment, only about 30% of hospitalized patients who have diarrhea will have CDAD.<sup>14</sup> Empiric therapy after submission of a stool specimen to the laboratory is appropriate for good patient management, but symptoms may resolve with cessation of the offending antimicrobial in 20% to 25% of patients. PMC can be diagnosed by direct visualization of pseudomembranes using lower gastrointestinal endoscopy (either sigmoidoscopy or colonoscopy). Direct visualization using either sigmoidoscopy or colonoscopy is required for the diagnosis of PMC, because no laboratory test is adequate for that specific diagnosis.<sup>14,15</sup> However, direct visualization using any of these techniques will detect pseudomembranes in only 51% to 55% of CDAD cases that are diagnosed by combined clinical and laboratory criteria that include both a positive culture for *C difficile* and a positive stool cytotoxin test.<sup>14,16</sup> Therefore, although endoscopy is required for the specific diagnosis of PMC, it is not sufficient to diagnose all cases of CDAD.

Since the original observations that *C difficile* toxins are responsible for antibiotic-associated colitis, several diagnostic tests have been developed that detect the cytotoxin (toxin B) and/or enterotoxin (toxin A) produced by *C difficile*.<sup>1</sup> Although the issue remains controversial, a combination of *C difficile* culture and one nonculture-based test (for toxin) has been recommended as the optimal laboratory approach.<sup>10,11,17-19</sup> Stool toxin testing alone also has been recommended.<sup>13,20,21</sup>

### **Specimen Collection and Transport**

The proper laboratory specimen for the diagnosis of *C difficile* -associated diarrhea is a watery or loose stool.<sup>10</sup> Except in rare instances when patients have ileus without diarrhea, swabs are unacceptable specimens because toxin testing cannot be done reliably on these specimens. Because 10% or more of hospitalized patients may be colonized with this organism,<sup>14</sup> evaluating a formed stool for the presence of *C difficile* or its toxins can decrease further the specificity of the diagnosis of CDAD. Specimens should be submitted in a clean, watertight container. Neither anaerobic transport nor the use of transport media are known to enhance recovery of either the organism or its toxin; therefore, the added cost of this type of transport is not justified. Processing one or two specimens from a patient at the

onset of a symptomatic episode usually is sufficient. If the initial stool sample is negative for toxin and *C difficile*, it may be useful to test additional diarrheal specimens. Testing three stools can increase the likelihood of a positive test by 10%<sup>22</sup>; however, this low increase in yield does not support routine testing of multiple stools as a cost-effective diagnostic practice.

### **Detection of *C difficile* Toxins**

**Cell Cytotoxicity.** Detection of toxin activity in stools from patients with antibiotic-associated colitis was the initial observation leading to the discovery of *C difficile* as the causative agent of this infection.<sup>23</sup> Virtually all *C difficile* produce either both toxins or neither toxin, permitting assay for cytotoxic activity as a reliable diagnostic test for CDAD, regardless of whether toxin A (enterotoxin) or toxin B (cytotoxin) is the primary toxin involved in the pathophysiology of this disease.<sup>24-27</sup> Numerous cell lines are satisfactory for detection of cytotoxin, most of which are readily available in virology laboratories,<sup>1</sup> or which may be purchased commercially in assay kits.<sup>28,29</sup> The age of the cell line used for testing may be important, particularly if cytotoxicity is to be detected at 4 hours. Tichota-Lee et al found that the most sensitive cell line for detecting toxin at low titer ( $\leq 1:160$ ) was human foreskin fibroblasts, and the least sensitive were 14-day-old HEp-2 cells.<sup>30</sup>

The dilution titer used for detection of a positive result by cytotoxin testing is extremely critical. Too low a titer will result in an unacceptably high rate of false-positive results, and too high a dilution will lead to low sensitivity.<sup>13</sup> When using HEp-2 cells, Shanholtzer et al screened for cytotoxicity at a specimen dilution of 1:40 (the final dilution in the assay well) and then performed a neutralization with antitoxin at a final dilution of 1:200.<sup>12</sup> When using MRC-5 cells, Peterson and Kelly screened for cytotoxicity at a specimen dilution of 1:200 and also performed the neutralization with antitoxin at a final dilution of 1:200.<sup>11</sup> Any stool specimen demonstrating cytotoxicity at the final titer (1:200), which was neutralized by antitoxin, was considered positive. Walker et al used a microtiter cytotoxicity test with dilutions of stool as low as 1:10.<sup>13</sup> Even at this low a dilution, their sensitivity of cytotoxin detection for patients with (very likely or probable) CDAD was 78% with a false-positive rate of only 5.9%. However, in this same study, their sensitivity of a tube cytotoxicity test was 67% with a false-positive rate of 26% using similar dilutions.<sup>13</sup> It is very difficult to test for cytotoxin at a final well dilution of  $\leq 1:10$  because most protocols describe a test sample:media ratio of 1:10, a dilution at which any stool may be toxic to many cell lines.

Using a combination of clinical and laboratory criteria to establish the diagnosis of CDAD, Gerding et al,<sup>14</sup> Peterson et al,<sup>31</sup> and Shanholtzer et al<sup>12</sup> have found the sensitivity of cytotoxin detection as a single test for the laboratory diagnosis of this illness to range from 67% to 100%. Compared to the isolation of toxigenic *C difficile*, Delmée et al also found the sensitivity of cytotoxin detection by cell culture to be relatively low at 71%.<sup>32</sup> The apparent suboptimal sensitivity of cytotoxin detection as a single test has been reported by others.<sup>14</sup> Recently, Barbut et al have used similar clinical criteria to that of Shanholtzer et al and found the sensitivity of culture to be 96%.<sup>12,33</sup> These data have led to the recommendation that culture should remain part of the diagnostic evaluation of this disease.<sup>11,17-19</sup>

**Immunoassay for Toxin A and/or B.** Several new commercial enzyme immunoassay (EIA) tests have been introduced that have undergone clinical trials reported in peer-reviewed papers. These include the Difco Cube test (Difco Laboratories, Detroit, MI) for toxin A,<sup>34,35</sup> the Premier EIA (Meridian Diagnostics, Cincinnati, OH) for toxin A,<sup>28,32,33,36-42</sup> the VIDAS CDA (BioMerieux Vitek, Inc, Hazelwood, MO) for toxin A,<sup>12,33,40,41</sup> the Cytoclone test (Cambridge Biotech Corp, Worcester, MA) for toxins A and B,<sup>33</sup> the TechLab Tox-A-Test (TechLab, Blacksburg, VA),<sup>39-41</sup> the Bartels Toxin A EIA, (Baxter Diagnostics, McGaw Park, IL)<sup>40</sup> and the CBC EIA test (Cambridge Biotech Corp, Worcester, MA) for toxins A and B.<sup>28</sup> When compared to diagnostic criteria that included a clinical definition of diarrhea, along with laboratory testing that included cytotoxin and culture, the sensitivity of these tests ranged from 63% to 94%, with a specificity of 75% to 100%. When culture was not included, the sensitivity ranged from 67% to 87%, with a specificity of 84% to 100%. When these tests were compared to cytotoxin results (obtained by cell culture assay) in the absence of clinical criteria, the sensitivity ranged from 71% to 99%, with a specificity of 92% to 100%. The Premier EIA test for toxin A has been studied most extensively and has demonstrated acceptable performance for toxin detection, with a mean test sensitivity of 77.5% (range, 65% to 88%) and a mean specificity of 98.6% (range, 95% to 100%).<sup>28,32,33,36-38</sup> Sensitivity of 87% to 99% compared to cell cytotoxicity has been found with the Tox-A-Test and Bartels, but specificity (92% to 96%) was lower than for the Premier EIA (97% to 100%).<sup>39-42</sup> Some of these new test methods also include a relatively large indeterminate or uninterpretable range of results that enhance the performance statistics but make the tests appear to be more clinically useful than they really are in the diagnostic laboratory.<sup>12,40</sup>

### Detection of the Organism

**Culture.** Along with cytotoxin detection, culture has been a mainstay in the laboratory diagnosis of CDAD and is essential for the epidemiologic study of nosocomial isolates. The description of an egg yolk agar base medium containing cycloserine, cefoxitin, and fructose (CCFA) by George et al provided laboratories with a selective culture system for recovery of *C difficile*.<sup>43</sup> Modifications have been suggested, such as decreasing the concentrations of cycloserine and cefoxitin by 50% to 250 and 8 µg/g of agar respectively.<sup>44</sup> Whereas this may enhance the growth of some *C difficile*, this modification also allows increased growth of other microbes, making the agar less selective. Shanholtzer et al have found the best performance using the original antibiotic concentrations described by George et al.<sup>45</sup> Quality control of CCFA media is acceptable when isolated colonies of American Type Culture Collection (ATCC, Rockville, MD) *C difficile* strains 9689 and 17858 both grow to at least 3 mm diameter in 48 hours.<sup>12</sup> Growth is enhanced on medium that has been reduced in an anaerobic environment prior to use. The strains must produce flat, yellow, ground-glass-appearing colonies with a surrounding yellow halo in the medium. Additionally, the Gram stain of these colonies must show typical morphology (gram-positive bacilli) for *C difficile*, and the medium must inhibit the growth of *Escherichia coli* ATCC 25922.<sup>12</sup> Other media, such as cycloserine-mannitol agar, cycloserine-mannitol-blood agar (CMBA), cycloserine-cefoxitin agar with various blood supplements, and media with alternative antimicrobial agents, have been evaluated.<sup>46,48</sup> The results of these investigations are not consistent. However, Mundy et al compared CCFA to CMBA using various incubation conditions. They found the best growth performance for clinical specimens was with the original formulation of CCFA using plates that were anaerobically reduced at least 4 hours before use.<sup>49</sup>

Marler et al found a significant variation in recovery rates of CCFA media (range, 46% to 81%) prepared by different manufacturers.<sup>50</sup> Shanholtzer et al also reported problems with CCFA performance in purchased media from five different suppliers during 5 of 6 years investigated.<sup>45</sup> Therefore, careful laboratory quality control of purchased differential and selective media for isolation of *C difficile* is required. The type of anaerobic environment (anaerobic chamber versus anaerobic jar versus anaerobic bag or pouch) does not affect recovery of *C difficile*.<sup>11</sup>

With experience, visual inspection of bacterial colonies that demonstrate typical morphology on agar and confirmation by Gram stain usually is sufficient for a presumptive identification of *C difficile*.<sup>51</sup> Isolates not fitting these criteria can be further identified

biochemically or by gas chromatography (GC). GC analysis has long been the accepted standard for identification and is the preferred method.<sup>52</sup> Several commercial identification kits have been evaluated for this use, and the RapID ANA (Innovative Diagnostic Systems, Inc, Atlanta, GA) appears to perform best.<sup>53-55</sup> If one of these commercial kits is used for identification of suspicious isolates, the results obtained should be comparable to those of GC or RapID ANA.

The diagnosis of CDAD in patients with diarrhea who have a negative stool toxin test but have *C difficile* recovered from their stool specimens by culture has been controversial. Because nontoxigenic strains are not considered pathogenic, the determination of in vitro toxin production by isolates cultured from toxin-negative stools may help somewhat to resolve this dilemma.<sup>12</sup> Toxin production in vitro was demonstrated in 73% of the *C difficile* isolates recovered from 264 patients with a clinical diagnosis of CDAD who had a positive stool culture but negative stool cytotoxin assay.<sup>56</sup> *C difficile* isolates from these patients produced lower cytotoxin titers in vitro than isolates from CDAD patients with positive stool cytotoxin assays, a possible explanation for the negative stool cytotoxin assay results. An alternative approach in this setting is to submit a new stool specimen to the laboratory for additional testing if the patient still is having diarrhea.

**Latex Test for *C difficile* Protein.** The Culturette Brand CDT Test (Becton Dickinson, Cockeysville, MD) is a latex agglutination test for detection of a *C difficile* -associated antigen. Initially, this test was believed to detect toxin A. However, subsequent studies have demonstrated that another clostridial protein, glutamate dehydrogenase, is detected.<sup>57,58</sup> The CDT test is perhaps the best studied, simplest, and most widely used alternative to culture or cytotoxin detection. The sensitivity and specificity of the available latex tests for the glutamate dehydrogenase antigen have varied widely. When compared to other tests in patients with clinical criteria for CDAD, the CDT test has a sensitivity of 58% to 68% and a specificity of 94% to 96%.<sup>12,31,38</sup> The latex test for *C difficile* -associated antigen is not sufficiently sensitive for the routine laboratory detection of *C difficile*, even though it is rapid, relatively inexpensive, and specific. Use of this test provides no information regarding the toxigenicity of the isolate, nor does it yield the isolate itself, which would be useful for epidemiologic investigations.

### **New Methodologies**

New tests based on detection of the toxin A and toxin B genes offer the potential for increased speed and sensitivity.<sup>59,60</sup> Polymerase chain reaction (PCR)

has been used for detection of toxigenic *C difficile*.<sup>61-63</sup> Amplification of a portion of either the toxin A gene,<sup>61,63</sup> the toxin B gene,<sup>64</sup> or both toxin A and toxin B genes has been performed.<sup>62</sup> The PCR protocol used by Kato et al amplified only toxin A from *C difficile* and gave no reaction when tested against two toxigenic *Clostridium sordellii*.<sup>61</sup> The method used by Gumerlock et al for detection of cytotoxin gene sequences directly in stool specimens was slightly more sensitive than detection of cytotoxin by cell culture.<sup>64</sup> In a series of 12 patients using this PCR approach, Kuhl et al detected toxigenic *C difficile* in the stool of four patients when toxin B was undetectable by the cytotoxin assay.<sup>65</sup> Application of these molecular diagnostic techniques in the clinical laboratory will require that they have improved sensitivity, specificity, and speed compared to culture and cytotoxin assay, and that they can be performed at a competitive cost.

### **Other Previously Used Test Methodologies**

Methods such as stool Gram stain, analysis for fecal leukocytes, counterimmunoelectrophoresis of stool specimens for toxin, and direct chromatography of stool for characteristic fatty acid chromatographic patterns of *C difficile* have been used with only marginal success.<sup>66-69</sup> They typically demonstrate low sensitivity and specificity. Gram stain for clostridia-like organisms and a smear for fecal leukocytes particularly are unrewarding, with a respective sensitivity for each test of 38% and 35% and a false-positive rate of 47% and 40%, respectively.<sup>68</sup> These methods are not comparable in sensitivity or specificity to either stool culture or cytotoxin detection by cell culture and should not be used as diagnostic criteria. Testing for blood in the stool is not helpful in the diagnostic evaluation for this infection, because only 26% (39 of 149 patients) were found to have blood in their stools when this was investigated.<sup>14</sup>

## **EPIDEMIOLOGY**

### **Historical Background**

The vast majority of anaerobic infections are considered to arise from endogenous sources. A number of important clostridial infections and intoxications, however, are caused by organisms acquired from exogenous sources. It is the ability of these organisms to produce spores that explains how *C difficile*, a fastidious organism in its vegetative state, can be acquired from outside the host. Ironically, *C difficile* first was described in 1935 as part of the "normal intestinal flora" of infants<sup>70</sup> and subsequently received little attention from microbiologists until the mid-1970s.<sup>71</sup>

In the mid-1970s, it was reported that colitis occurred in 10% of clindamycin-treated patients.<sup>15</sup>

Although PMC had been recognized as early as 1893,<sup>72</sup> the increased frequency of PMC and its obvious association with antimicrobial therapy prompted investigations that uncovered the role of *C difficile*.<sup>73</sup> *C difficile* is now recognized as the primary pathogen responsible for antibiotic-associated colitis and 15% to 25% of cases of antibiotic-associated diarrhea.<sup>20</sup>

*C difficile* can be detected in stool specimens of many healthy children<sup>75</sup> and some adults.<sup>76,77</sup> Although these data supported the potential for endogenous sources of human infection, there was early circumstantial evidence to suggest that this pathogen could be transmissible and acquired from external sources. Cases often appeared in clusters and outbreaks within institutions.<sup>78,79</sup> Animal models of disease also provided evidence for transmissibility of *C difficile*.<sup>80,81</sup> Subsequently, many epidemiologic studies of CDAD have confirmed the importance of *C difficile* as a nosocomial pathogen.<sup>6,7,14</sup>

### Prevalence

Prevalence of *C difficile* colonization or carriage varies markedly, depending on the population and the setting. In some populations—newborn infants, for example—asymptomatic carriage is common. Pediatric carriage of *C difficile* is influenced strongly by the child's age, being highest in young infants and declining markedly after the first year.<sup>75</sup> Although carriage is much higher in infants, overt disease in this group is much less frequent than in adults. Studies of healthy adults have reported a wide range of asymptomatic carriage rates from 2% in Sweden<sup>76</sup> to 15% in Japan.<sup>77</sup> Approximately 20% of initially culture-negative adult patients were reported to acquire the organism during hospitalization in one institution with a very high prevalence of CDAD; two thirds of those patients remained asymptomatic carriers.<sup>6</sup>

Prevalence of *C difficile* in symptomatic (diarrhea) patients has been examined frequently in hospital settings. In one hospital, 30% of adult patients who developed diarrhea during hospitalization were found to have this organism.<sup>14</sup> Another study determined that diarrhea occurring in adult patients after 72 hours of hospitalization almost always was due to *C difficile*, if it was due to any recognized infectious agent, suggesting that evaluation for other enteric pathogens was much less cost-effective.<sup>4,5</sup> However, *C difficile* continues to be an uncommon pathogen in other institutions, a finding that is not fully explained by differences in diagnostic methodologies.

Until recently, most studies of prevalence have focused on patients hospitalized on acute-care hospital wards. However, *C difficile* clearly is important for other patient populations. For some patients, such as

those with inflammatory bowel disease who also receive antimicrobial agents,<sup>82</sup> the role of *C difficile* as a pathogen may be especially hard to evaluate. *C difficile* has been found frequently in patients from some chronic-care facilities. In this more debilitated population, it may cause greater morbidity and mortality than in other settings.<sup>83</sup> Among patients in two "freestanding" rehabilitation hospitals who had enteric evaluations for diarrhea, 25% were found to have positive diagnostic tests for *C difficile*.<sup>84</sup> Community-acquired CDAD cases also are recognized, but the incidence is low (<1 case per 10,000 antibiotic prescriptions reported from one large outpatient setting<sup>85</sup>). The relative importance of *C difficile* as a pathogen in developing countries is not completely defined, but the widespread, uncontrolled use of antimicrobial agents in many of these countries may play an important role in the epidemiology of CDAD in this setting.<sup>86,87</sup>

### Risk Factors

Exposure to antimicrobial agents has been identified as the preeminent risk factor for developing disease due to *C difficile*. Agents that are active against anaerobic bacteria are considered to present the greatest risk, presumably because of their ability to alter intestinal microecology. Although clindamycin was linked most closely with the disease historically<sup>14,15,88</sup> and still carries one of the highest relative risks, more cases at present are attributed to therapy with  $\beta$ -lactam agents because of their common use.<sup>20</sup> Duration of antecedent therapy may be brief, including short courses given for surgical prophylaxis.<sup>89,90</sup> However, it also has been shown that receipt of multiple antimicrobial agents for therapy of infection puts patients at higher risk than brief use for prophylaxis.<sup>14</sup> Precipitating agents also include cancer chemotherapy drugs that have antimicrobial activity, even though not used primarily for that purpose.<sup>91</sup>

Gastrointestinal surgery itself and other types of gastrointestinal manipulations have been associated with increased risk of disease.<sup>92</sup> Other reported risk factors include older age and "excess antibiotic use."<sup>76,89,93</sup> In some studies, patients who are more seriously ill appear to be more likely to develop CDAD.<sup>93</sup> Although *C difficile* usually is not considered to be an opportunistic pathogen, severe, even fatal, disease may occur more frequently in some compromised hosts.<sup>94</sup> Certain compromised hosts, such as those infected with the human immunodeficiency virus or patients who have received bone marrow transplants, may acquire strains that are different from other hosts.<sup>95,96</sup> However, in addition to strain-related characteristics, host factors also contribute to the outcome of *C difficile* acquisition, although the

exact nature of these host factors is poorly defined.<sup>97</sup> Host factors that may explain the high frequency of colonization and low incidence of disease among patients with cystic fibrosis<sup>98</sup> and in newborn infants<sup>78</sup> have not been explored fully. Few studies have examined specific characteristics of the host, such as immune response.<sup>99-102</sup>

Obviously, for a pathogen to be acquired from an exogenous source, exposure to such a source is a crucial risk factor. Thus, admission to a hospital with a high endemic level of CDAD or during an outbreak would constitute a risk factor in itself. Hospitals with high rates of CDAD have been found to have predominant single strains of *C difficile* causing disease or to have a diverse population of *C difficile* strains present.<sup>6,7,88,103</sup> Indeed, it sometimes is difficult to distinguish between a clinical relapse and a new infection that results from continued or repeated exposure to an external source.<sup>104,105</sup>

### Routes of Transmission

Although sporadic cases of CDAD occur in non-hospitalized patients, most cases clearly are the result of nosocomial transmission. In one study, patients who were culture positive at the time of hospital admission were most likely to have had prior exposure to that same hospital.<sup>106</sup> Other studies using sensitive typing methods have documented nosocomial transmission in both endemic and epidemic settings.<sup>6,107,108</sup>

The two major potential reservoirs of *C difficile* in hospitals are infected humans (symptomatic or asymptomatic) and inanimate objects. Patients with symptomatic intestinal infection probably are the major reservoir. Careful studies have indicated that asymptomatic colonization is remarkably common in hospitals with a high prevalence of symptomatic disease. One study detected carriage of *C difficile* in 20% of randomly selected and case-matched control adult patients.<sup>14</sup> Admission to another hospital in which *C difficile* was endemic resulted in nosocomial acquisition for 20% of patients who initially were culture negative; of those patients, two thirds were asymptomatic.<sup>6</sup> The rate at which patients acquire *C difficile* in-hospital was shown to be linear (8% per week) on one well-studied ward of medical and surgical patients.<sup>106</sup> In these and other studies, many of these asymptomatic individuals could be implicated as the source of the strain recovered from other patients who developed symptomatic disease.<sup>6,7,106</sup> Intestinal infection of healthcare workers also could provide a reservoir of *C difficile*, but there has been little evidence to indicate that this is an important concern.<sup>14</sup>

It is far more likely that healthcare workers contribute to transmission because of transient hand carriage.<sup>6,8</sup> Reduction in CDAD rates associated with

use of gloves provides strong support for the importance of hand carriage.<sup>109</sup> Personnel hand carriage probably accounts for the majority of hospital transmission of *C difficile*.

Contamination of environmental surfaces in the hospital has been well documented.<sup>8,110,111</sup> Environmental contamination is due to the persistence of spores, which can be highly resistant to cleaning and disinfection measures. Contamination has been found to be most extensive in close proximity to symptomatic patients who are likely to soil their immediate surroundings, and it can persist for many weeks or months after the patient has left the environment. Whether environmental contamination has a direct role in transmission is not clear, although transfer to hands could occur when contaminated surfaces are touched. In this way, the hands of the patients or of their healthcare workers could become transiently colonized. In one study, the same strains were found on environmental surfaces in hospital rooms and in cultures of patients in those rooms who subsequently became infected or colonized.<sup>6</sup> Another investigation, however, found that strains that were recovered from patients and their hospital environments were not the same,<sup>2</sup> suggesting a less important role for environmental contamination. Caution in interpreting environmental contamination is required, because multiple *C difficile* strains may be present in the environment, and extensive typing of all environmental isolates is required for comparison with patient isolates.

Direct exposure of patients to certain contaminated items in hospitals may be important in transmission. Contaminated commodes, bathing tubs for neonates, telephones, and rectal thermometers have been implicated as potential sources of *C difficile*.<sup>78,112-115</sup> Other potential sources of *C difficile* have been identified but have not been implicated in transmission to humans. Although there have been rare reports of contaminated food products,<sup>116</sup> evaluations of hospital foods and food preparation areas failed to detect the organism.<sup>117,118</sup> Various animals also have been found to harbor *C difficile*, including horses, cats, and dogs.<sup>119</sup> These household pets could constitute a reservoir, but a recent study indicated that their strains were different from those recovered from humans.<sup>120</sup>

In addition to the convincing evidence for horizontal transmission in the hospital setting, there has been some suggestion that "vertical" (maternal-child) transmission might occur. As noted earlier, neonates frequently experience asymptomatic acquisition of *C difficile*. Hafiz reported that 71% of women attending a clinic for sexually transmitted disease had positive vaginal cultures,<sup>121</sup> but other investigators could not confirm this finding and provided alternative evidence

**TABLE 2**  
METHODS USED TO TYPE *CLOSTRIDIUM DIFFICILE* (WITH REFERENCES)

| Phenotypic  | Genotypic   |
|---|---|
| PAGE <sup>27,128</sup>  | Plasmid typing ± REA <sup>108</sup>                 |
| PAGE with [ <sup>35</sup> S]methionine labelling <sup>126</sup> | Total genomic REA typing <sup>105,123</sup>         |
| PAGE with immunoblotting <sup>127-129</sup>                     | Ribosomal RNA probe analysis <sup>123,133,134</sup> |
| Serotyping <sup>128,130</sup>                                   | Pulsed-field gel electrophoresis <sup>134</sup>     |
| Bacteriophage/bacteriocin typing <sup>131,132</sup>             | PCR with arbitrary primers <sup>135-137</sup>       |
| Antimicrobial susceptibility testing <sup>79</sup>              |   |

Abbreviations: PAGE, polyacrylamide gel electrophoresis; REA, restriction endonuclease analysis; PCR, polymerase chain reaction.

for infant acquisition from other external sources.<sup>122</sup> The data suggest that the same modes of nosocomial transmission are operative in the neonatal unit as in other parts of the hospital.<sup>78,122</sup>

### Typing

The development and application of new methods that allow the typing of, or discrimination between, different strains of *C difficile* have been crucial for defining the epidemiology of CDAD. It is important to note that many epidemiologic studies can be conducted only if there are organisms available for typing. Even though culture for *C difficile* is somewhat difficult and often not available in many laboratories, obtaining of cultures is essential for most epidemiologic investigations. Typing methods can be used to identify different strains and thus allow investigations of endemic disease as well as outbreaks. Modes of transmission, as well as efficacy of interventions, can be examined. Clinical issues also can be addressed by typing. The question of whether so-called relapses of diarrhea actually represent failures to eradicate the initial infecting strain or, instead, are new infections with new strains has been examined by using typing methods. It has been shown that reinfections with new strains occur more commonly than expected.<sup>104,105</sup>

A wide variety of methods have been used for typing of *C difficile* (Table 2); but, as yet, there are relatively few comparisons of these different methods to determine their relative merits.<sup>123,124</sup> An optimal typing method should be reproducible (giving the same results each time the same organism is tested), should be able to type all isolates, and should have high discriminatory power (ie, should be capable of distinguishing among many different organisms). Ideally, the method also would be inexpensive, technically simple, and readily available. Many methods

have been described, but no single method can yet be said to have all of the desirable features mentioned.

Among typing methods employing phenotypic characteristics of *C difficile*, susceptibility testing was exploited early on and provided some of the first evidence for nosocomial spread of the organism in a London, England, hospital.<sup>79</sup> However, antibiograms have limited usefulness, as the MICs of most antimicrobials for *C difficile* fall within a narrow range.<sup>125</sup> Several electrophoretic methods based on cellular and surface protein patterns have been developed into very useful investigative tools; these include polyacrylamide gel electrophoresis combined with radiolabeling<sup>126</sup> or with immunoblotting.<sup>127-129</sup> A rapid serotyping system using slide agglutination also has been used successfully and recently has been refined by the removal of cross-reacting flagellar antigens.<sup>139</sup> A system based on susceptibilities of *C difficile* to bacteriocins and bacteriophages has been developed and exploited for epidemiologic studies; but, some strains are not typeable, and the method is not available widely.<sup>131,132</sup>

Genotypic typing methods have a theoretical advantage over methods dependent on phenotypic expression of antigens that in some cases are not stable or subject to culture conditions. Examination of plasmid DNA as "plasmid fingerprints" or with restriction endonuclease analysis (REA) has been of some value but cannot be used to type organisms lacking plasmids.<sup>108</sup> REA of total genomic DNA using the restriction enzyme *Hind*III has proven to be an excellent system capable of a very high degree of discrimination.<sup>105,123</sup> The main disadvantage of REA is that comparison of complex DNA fragment patterns usually requires comparing isolates that are run on the same gel, and comparison of isolates from different institutions requires maintenance of a large type library.<sup>123</sup> In addition, plasmid preparations restricted with the same enzyme may be required to resolve band differences in the whole DNA preparations. Refinements of REA that simplify the banding patterns include the use of ribosomal RNA probes<sup>123,133</sup> and DNA probes of other gene sequences.<sup>125</sup> Although easier to interpret, these techniques have considerably less discriminating power than conventional REA.<sup>123,134</sup> Another modification of REA that is being developed employs pulsed-field gel electrophoresis in conjunction with restriction enzymes that generate fewer (and larger) DNA fragments. This method is highly sensitive, but approximately 5% of isolates cannot be typed due to DNA degradation.<sup>134</sup> Polymerase chain reaction technology also is being evaluated for typing of *C difficile*, but no large-scale clinical isolate comparisons have been made to date.<sup>135-137</sup>

TABLE 3  
INFECTION CONTROL MEASURES FOR THE PREVENTION OF  
HORIZONTAL TRANSMISSION OF *CLOSTRIDIUM DIFFICILE*

|  | Intervention |             |
|--|--------------|-------------|
|  | Efficacy*    | Reference   |
| 1. Barrier precautions   |              |             |
| a. Glove use   | Proven       | 110         |
| b. Handwashing   | Probable     | 6,138       |
| c. Private room or cohorting                                   | Probable     | 83,139,143  |
| d. Gowns   | Untested     |             |
| 2. Environmental cleaning, disinfection, or use of disposables |              |             |
| a. Rooms   | Possible     | 142,146,147 |
| b. Commodes  | Untested     |             |
| c. Rectal thermometers   | Proven       | 113         |
| d. Endoscopes  | Probable     | 144,145     |
| 3. Identification and treatment of asymptomatic carriers       |              |             |
| a. Vancomycin treatment  | Possible     | 147,152     |
| b. Metronidazole treatment                                     | Ineffective  | 83,152      |
| c. Identification and isolation or cohorting of carriers       | Untested     | 106         |

\* Proven: published efficacy in reducing incidence of CDAD when employed in the healthcare setting. Probable: accepted practice without specific published evidence of efficacy with CDAD. Possible: some published evidence of use, but efficacy inconclusive. Ineffective: published evidence of lack of efficacy in a healthcare setting. Untested: no published human efficacy data.

## PREVENTION AND CONTROL

In considering the possible avenues of infection control, it is useful to examine two approaches that are distinct but related, namely (1) efforts directed at interrupting horizontal spread of *C difficile* (to keep the patient from acquiring the organism), and (2) efforts to minimize the possibility that organism exposure will result in clinical infection (reducing individual risks of disease; Tables 3 and 4). Given the large number of options in the tables, it should be obvious that no one method has been uniformly effective in interrupting nosocomial spread and that there may not be a single method that is effective in control of this problem. Rather, different methods may be more or less effective in different institutions, depending on the local epidemiology and the ability to employ the proposed methods successfully. It also is likely that unknown epidemiologic factors are at work that have not been recognized and for which new and innovative prevention and control measures will be required.

### Barrier Methods

The rationale for the use of these methods is that *C difficile* is spread from patient to patient by direct

TABLE 4  
INFECTION CONTROL MEASURES TO REDUCE THE RISK OF  
*CLOSTRIDIUM DIFFICILE* DIARRHEA

|  | Intervention |           |
|--|--------------|-----------|
|  | Efficacy*    | Reference |
| 1. Antimicrobial usage restriction                             | Proven       | 88,140    |
| 2. Prophylactic treatment of patients receiving antimicrobials |              |           |
| a. <i>Saccharomyces boulardii</i>                              | Possible     | 154       |
| b. <i>Lactobacillus</i> species                                | Untested     | 159,160   |
| c. <i>Clostridium difficile</i> antibody                       | Untested     | 155       |

\* Proven: published efficacy in reducing incidence of CDAD when employed in the healthcare setting. Probable: accepted practice without specific published evidence of efficacy with CDAD. Possible: some published evidence of use, but efficacy inconclusive. Ineffective: published evidence of lack of efficacy in a healthcare setting. Untested: no published human efficacy data.

contact or by contact with hospital personnel, presumably via their hands. There is ample evidence for personnel hand contamination,<sup>6,8</sup> but this is not a uniform finding, even in institutions with a high rate of CDAD.<sup>14,109</sup> Indirect evidence of the importance of personnel hand carriage is inferred from a prospective controlled trial of vinyl glove use for handling body substances, which showed a significant decline in CDAD rates from 7.7 cases per 1,000 discharges before glove use to 1.5 per 1,000 after institution of glove use ( $P = .015$ ).<sup>109</sup> Good handwashing practice also should be effective in preventing *C difficile* transmission, but McFarland and colleagues<sup>6</sup> observed that *C difficile* persisted on the hands of 14 (88%) of 16 personnel who had washed with nondisinfectant soap. Washing with a disinfectant (4% chlorhexidine gluconate) reduced the positive culture rate to 14% (1 of 7 personnel). One study of experimental hand seeding of *C difficile* showed no difference between soap and chlorhexidine gluconate in removing *C difficile* from hands.<sup>138</sup> Neither of these observations have been verified clinically; the emphasis should be on compliance with the use of handwashing (with either a disinfectant or soap) until more data are available in the clinical setting to support one handwashing agent over another.

The use of isolation techniques (enteric isolation, private rooms, and cohorting of infected patients) has been employed for outbreak control with varied success.<sup>83,139-141</sup> These techniques usually are employed simultaneously with other measures, such as antimicrobial control or environmental cleaning, which makes evaluation of the benefit of isolation difficult. These measures are based on the premise that patients with active CDAD are the primary source for spread of disease within the

institution. Struelens and colleagues demonstrated successful reduction in new *C difficile* cases by using an aggressive policy of increased cultures of diarrheal stools for *C difficile*, early enteric isolation and treatment of CDAD patients with vancomycin, and daily room environmental cleaning with a disinfectant solution of 0.04% formaldehyde and 0.03% glutaraldehyde.<sup>142</sup> In this institution, with a relatively low rate of CDAD (1.5 cases per 1,000 discharges), there was further reduction to 0.3 cases per 1,000 discharges. Placing the focus for control measures on clinically ill patients (those with CDAD) was successful in this institution, supporting the hypothesis that patients with diarrhea, who are known to have the highest number of organisms in their stools and in their immediate hospital environment, are the most likely source of nosocomial spread. In institutions with higher rates of CDAD (7.8 to 22.5 per 1,000 discharges), the number of asymptotically colonized patients has been found to be considerably higher than the number with CDAD, and these patients have been postulated also to be a source of *C difficile* spread.<sup>6,7,140</sup> Whether focusing control measures on symptomatic patients alone in these higher-risk institutions also would be effective is not known, nor is it known if it is necessary to place all patients with CDAD in private rooms or on enteric precautions, or if it is sufficient to do so only for those patients who are incontinent or unable to exercise good bowel hygiene. Both approaches have been used, but there are no comparative data for the relative efficacy of either approach.<sup>143</sup> It is possible that both strategies may be effective and that practical considerations should dictate which is used. For example, hospitals in which *C difficile* is a common endemic pathogen may find it impossible to provide a private room for every infected patient, whereas in a setting in which CDAD is rarely found, aggressive isolation may be the optimal way to control spread.

### **Environmental Cleaning and Disinfection**

Evidence for contamination of the hospital environment by *C difficile* is compelling.<sup>6,8,110,118</sup> Virtually any room surface may be contaminated, and the rate of environmental contamination rises in proportion to the status of the patients in the area, being lowest (<8%) for rooms of culture-negative patients, intermediate (8% to 30%) for rooms of asymptomatic *C difficile*-colonized patients, and highest (9% to 50%) for rooms of patients with CDAD.<sup>6,118</sup> Environmental contamination has been linked to spread of *C difficile* via a contaminated commode chair,<sup>112</sup> a nursery baby bath,<sup>78</sup> and contaminated electronic rectal thermometer handles.<sup>113,114</sup> In one hospital, replacement of

electronic rectal thermometers with single-use disposable thermometers was associated with a significant reduction in the incidence of CDAD.<sup>113</sup>

Fortunately, the risk of transmission via contaminated endoscopes appears to be low if scopes are properly cleaned and disinfected using 2% alkaline glutaraldehyde immersion for as little as 5 minutes.<sup>144</sup> In vitro testing of glutaraldehyde preparations also indicates that exposure of *C difficile* spores to 2% alkaline glutaraldehyde for 10 minutes is sporicidal.<sup>145</sup> Endoscopes have not been implicated in the transmission of *C difficile*, but the potential for spread via this mechanism is preventable by careful cleaning, disinfection with 2% alkaline glutaraldehyde immersion, and prompt drying using forced air.<sup>144</sup>

Disinfection is effective in reducing the number of *C difficile* -positive cultures from the environment.<sup>146,147</sup> Kaatz and colleagues used unbuffered hypochlorite (500 ppm available chlorine) to disinfect the ward environment during a small *C difficile* outbreak and reduced the ward contamination rate from 31.4% to 16.5% of sampled sites.<sup>146</sup> Phosphate-buffered hypochlorite (1,600 ppm available chlorine) was even more effective and resulted in a 98% reduction in surface contamination when used in one room. The hypochlorite disinfection intervention was made following the fifth case in a seven-patient outbreak of CDAD (only two cases occurred after intervention), leaving some questions as to its efficacy due to the limited number of cases. Extensive disinfection (and painting and renovation) was done on a leukemia unit and resulted in a marked decline in the number of positive environmental cultures and the number of new *C difficile* patient acquisitions.<sup>147</sup> Because all patients (symptomatic and asymptomatic) who harbored *C difficile* were treated with vancomycin, it is not possible to determine the role played by environmental disinfection in reducing *C difficile* cases in this study. Struelens et al<sup>142</sup> used 0.04% formaldehyde and 0.03% glutaraldehyde disinfection and were able to show a reduction in environmental contamination from 13% to 3% ( $P = .04$ ). There was an associated decline in new cases of CDAD, but a program of increased culturing of stools for *C difficile*, enteric isolation, and treatment of positive patients also was instituted. Well-controlled trials of environmental disinfection are needed to determine if there is a benefit from this procedure, and the optimal agents to use in controlling nosocomial *C difficile* transmission.

### **Identification and Treatment of Asymptomatic Patient Carriers**

As mentioned above, Delmée and colleagues demonstrated a significant reduction in new *C difficile* infections in a leukemia unit when combining oral

vancomycin treatment of asymptotically colonized patients (500 mg 4 times daily for 7 days) with extensive environmental renovation and cleaning.<sup>147</sup> The rationale for treating these asymptomatic patients is that they serve as a reservoir for horizontal spread of *C difficile* to other patients, via either the environment or the hands of medical personnel. It is not clear if the asymptomatic patients are at increased risk of CDAD themselves. Two studies have failed to show any increased risk of CDAD in asymptotically colonized patients when compared to patients who are not colonized with *C difficile*.<sup>7,48</sup>

In contrast to vancomycin, metronidazole was ineffective in reducing the incidence of new CDAD cases when administered to all *C difficile* carriers in a chronic-care facility.<sup>83</sup> In addition to metronidazole (dose and duration not stated), enteric precautions and antimicrobial use limitations also were employed. Metronidazole (500 mg 3 times daily for 10 days) also was used to treat CDAD cases and asymptomatic *C difficile* carriers in a nursing home.<sup>149</sup> New *C difficile* cases did not occur after this intervention, but only five asymptomatic patients were treated, two of whom died during follow up.

Metronidazole is absorbed completely and cannot be detected in stools in the absence of diarrhea.<sup>150,151</sup> One prospective trial showed no significant reduction of *C difficile* carriage following oral metronidazole when compared with placebo treatment, whereas nine of 10 patients treated with vancomycin became culture negative on treatment.<sup>152</sup> At 70 days of follow up, however, four of six patients who cleared on vancomycin again were positive for *C difficile* (including one patient who developed CDAD), whereas only one of nine placebo-treated patients remained positive ( $P < .05$ ). Thus, treatment of asymptomatic *C difficile* carriers is effective only for vancomycin, but patients treated with vancomycin may be at increased risk for prolonged carriage after treatment is stopped. The efficacy of vancomycin treatment of asymptomatic carriers as a control measure to interrupt hospital transmission is unclear. Similarly, it has been suggested that identification of asymptomatic carriers and institution of more stringent barrier precautions may be useful in interrupting an outbreak, but there are no available data to support such a measure.<sup>106</sup>

### **Antimicrobial Use Restriction**

The prior exposure of patients with CDAD to antimicrobials is virtually a universal precondition for the disease. Antimicrobial use is very common in all hospitalized patients; 82% of 108 CDAD case-matched control patients in one study had received antimicrobials within the previous 14 days.<sup>14</sup> Specific agents such as clindamycin, ampicillin, amoxicillin, or cepha-

losporins most often are associated with increased risk of CDAD.<sup>14,153</sup> The number of antimicrobials administered, number of doses, number of antibiotic days, and use of antibiotics for infection treatment (rather than prophylaxis) have been associated with increased risk of CDAD.<sup>14,89,93,140</sup>

Limitation or restriction of use of agents found to be associated with increased CDAD rates is an intuitively attractive approach to reduction of cases, yet there are few reports that demonstrate successful implementation of this approach. Clindamycin restriction has been studied the best. Brown and colleagues<sup>140</sup> instituted restriction of clindamycin first by voluntary educational means, then by mandatory infectious disease approval restriction, and showed a decline in CDAD rates from 22.5/1,000 discharges to 7.4/1,000 over a 1-year period, paralleling the decline in clindamycin usage. Additional control measures included early institution of enteric precautions and early empiric treatment of suspected CDAD. Declines in ampicillin, cephalosporin, and aminoglycoside use also were noted during the same time period. Pear and colleagues,<sup>88</sup> in a year-long hospital outbreak of CDAD, identified clindamycin use as a risk factor, effectively curtailed its use, and demonstrated a significant decrease in new cases of CDAD within 3 months.

### **Prophylaxis of Patients Receiving Antimicrobials**

Because it is difficult to avoid antimicrobial use in hospitalized patients, there is considerable interest in the use of prophylactic agents that could reduce the risk of CDAD in patients who are receiving antimicrobials. Several agents have been proposed for this purpose, including *Saccharomyces boulardii*, lactobacilli of various types, and orally administered *C difficile* antibodies.<sup>154,155</sup> The best-studied agent in humans is the yeast *S boulardii*.<sup>154</sup> In a prospective, blinded study, this organism was found to reduce antibiotic-associated diarrhea significantly ( $P = .038$ ) when given as 1 g of live lyophilized yeast orally per day during antibiotic administration and for 2 weeks afterward. The incidence of CDAD was reduced from 31% (5 of 16) in placebo patients to 9.4% (3 of 32) in *S boulardii* recipients, but this was not statistically significant ( $P = .07$ ).<sup>154</sup>

*C difficile* antibodies produced in cattle and extracted from colostrum have been orally administered prophylactically to hamsters and have been shown to protect these animals from *C difficile* disease.<sup>155</sup> No data are available to date for human use of these bovine *C difficile* antibodies, but similar bovine antibodies to rotavirus and toxigenic *E coli* have been shown to be protective against these organisms when administered prophylactically to humans.<sup>156,157</sup> Simi-

larly, lactobacillus preparations in the form of yogurt or acidophilus milk have been used to treat recurring *C difficile* diarrhea and to reduce diarrheal side effects of antibiotics.<sup>158-160</sup> At this time, no data are available for the efficacy of lactobacilli as specific prophylactic agents for the prevention of CDAD in humans, but there was no protection from disease in the hamster model.<sup>161</sup>

### GENERAL TREATMENT PRINCIPLES

Effective therapeutic strategies exist for the majority of patients who develop CDAD. However, prior to initiating specific therapy, it should be kept in mind that CDAD will resolve in 23% of patients within 2 to 3 days of discontinuing the offending antimicrobial<sup>162</sup> and that specific anti-*C difficile* treatment incurs a risk of relapse following treatment completion.<sup>163</sup> The following general principles should guide treatment of CDAD.

First, if possible, the offending antimicrobials should be discontinued or substituted with an antimicrobial that is less predisposing to CDAD (eg, metronidazole, vancomycin, an aminoglycoside, or possibly a quinolone).

Secondly, the specific therapy should be administered orally, particularly in the case of vancomycin. Although there is anecdotal experience using intravenous metronidazole in the treatment of CDAD,<sup>164</sup> and bactericidal fecal concentrations can be achieved in patients with acute disease when metronidazole is administered by this route,<sup>165</sup> all proven therapies have been with oral regimens.

Third, nearly all patients respond to specific therapy with vancomycin or metronidazole even after relapses following specific treatment with the same drug.<sup>143</sup> However, patients with toxic megacolon or ileus may require treatment by routes other than, or in addition to, oral administration.<sup>143</sup> Although most patients with CDAD show some improvement within the first 2 days of initiating treatment, the mean time to resolution of diarrhea ranges from 2 to 4 days,<sup>162,166</sup> and some patients respond more slowly to specific therapy. Patients should not be deemed therapeutic failures until at least 6 days of treatment have been given.

Fourth, treatment is more likely to be successful if continued for 10 days. Vancomycin given at a dosage of 125 mg four times daily for 5 to 7 days appears to be less efficacious than when given for at least 10 days.<sup>166</sup>

Fifth, antiperistaltic agents should not be administered, either alone or in conjunction with specific therapy. Anecdotal reports indicate that phenoxylate-atropine (Lomotil, G.D. Searle and Co, Chicago, IL) is deleterious to patients with CDAD<sup>167</sup> and may predispose them to toxic megacolon.<sup>168</sup> Theoretically, it also

could lead to increased absorption of metronidazole by reducing diarrhea and potentially could cause failure of metronidazole treatment. Finally, test-of-cure cultures or toxin assays following treatment are not recommended, as they are imperfect predictors of subsequent relapse.<sup>169</sup>

### Specific Therapies

Most experience in specific treatment of CDAD has been with metronidazole and vancomycin. Important theoretical considerations for anti-infective therapy include in vitro susceptibility of *C difficile* to the antimicrobial and the concentration of that antimicrobial at the site of infection. Both metronidazole and vancomycin are highly active in vitro with a MIC<sub>90</sub> of 0.4 and 1.6 µg/mL, respectively.<sup>166</sup> Fecal drug concentrations in the range of 2,000 to 5,000 µg/mL (several log<sub>10</sub> higher than the MIC for *C difficile*) can be achieved with vancomycin, which is poorly absorbed.<sup>170</sup> The concern with metronidazole has been that, unlike vancomycin, it is well absorbed, and fecal concentrations are low or absent in healthy volunteers<sup>150,151</sup> and asymptomatic *C difficile* carriers.<sup>152</sup> However, bactericidal fecal concentrations were detected in all of the acute specimens obtained from nine patients with CDAD with a mean (± standard deviation [SD]) concentration of 9.3 ± 7.5 µg/gm stool (wet weight).<sup>165</sup> Metronidazole fecal concentrations decreased as diarrhea improved, suggesting that, during illness, the drug may be secreted directly through inflamed colonic mucosa or that decreased intestinal transit time with diarrhea results in decreased absorption.<sup>171</sup>

Despite theoretical concerns about achieving significant fecal levels, metronidazole is proven effective treatment for CDAD. A clinical cure rate of 98% has been compiled for metronidazole, as previously reviewed in 1989 for all reported therapeutic trials of CDAD, including 445 metronidazole-treated episodes,<sup>166</sup> and as reported in 1994 for 632 patients treated in one hospital.<sup>143</sup> Metronidazole also has been compared to vancomycin in a prospective randomized trial of 94 patients with CDAD, 33 of whom had documented pseudomembranous colitis<sup>162</sup> (Table 5). In this study, treatment failure rates (4.7% and 0%) and relapse rates (4.7% and 11.5%) were similar for metronidazole and vancomycin, respectively. Further experience with metronidazole in the treatment of 632 patients with CDAD from this same institution documents a drug intolerance rate, treatment failure rate, and relapse rate of 1%, 2%, and 7%, respectively.<sup>143</sup> Metronidazole also is the least expensive treatment for CDAD, with a pharmacy cost of 68 cents for a 10-day treatment course (250 mg four times daily) compared to vancomycin at \$100 (125 mg oral pulvule

**TABLE 5**  
**SUMMARY OF RANDOMIZED, COMPARATIVE TRIALS OF ORAL THERAPY FOR *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA\***

| Antibiotic    | Regimen             | Patients Studied | Cure      | Relapse  | Mean Days to Resolution | References  |
|---------------|---------------------|------------------|-----------|----------|-------------------------|-------------|
| Metronidazole | 250mgid x 10d       | 42               | 40 (95%)  | 2 (5%)   | 2.4                     | 162         |
| Vancomycin    | 500 mg qid x 10d    | 87               | 87 (100%) | 13 (15%) | 2.6-3.6                 | 162,175,176 |
|               | 125mgid x 7d        | 21               | 18 (86%)  | 6 (29%)  | 4.2                     | 177         |
|               | 125mgid x 5d        | 12               | 9 (75%)   | ?        | <5                      | 178         |
| Teicoplanin   | 100 mg bid x 10d    | 26               | 25 (96%)  | 2 (8%)   | 3.4                     | 175         |
| Bacitracin    | 20,000-25,000 U qid | 36               | 28 (78%)  | 10 (28%) | 2.5-4.1                 | 176,177     |
|               | x 7-10d             |                  |           |          |                         |             |
| Colestipol    | 10 gm qid           | 14               | 5 (36%)   | ?        | <5                      | 178         |
| Placebo       | —                   | 14               | 3 (22%)   | ?        | <5                      | 178         |

\* Adapted from Reference 166.

four times daily) or \$115 (500 mg intravenous preparation given orally four times daily) (each based on Minneapolis, MN, VA Medical Center Pharmacy cost).<sup>143</sup> Therefore, with the exception of a small number of patients who cannot tolerate the medication or who don't respond, metronidazole is an efficacious and inexpensive treatment for CDAD, although it does not have a US Food and Drug Administration indication for this use.

Vancomycin was the first agent demonstrated to be highly effective for CDAD and is the drug to which all subsequent therapies have been compared.<sup>172</sup> All patients treated in comparative trials with vancomycin at a dosage of 500 mg four times daily for 10 days have had resolution of diarrhea (Table 5). This response rate drops to 75% when the regimen is decreased to 125 mg four times daily for 5 days (Table 5). However, there were no treatment failures when the lower dose vancomycin regimen (125 mg four times daily) was given for 10 days.<sup>173</sup> Despite remarkable efficacy, 15% of treated patients relapse. Recommendations by the Hospital Infection Control Practices Advisory Committee (HICPAC) for Preventing the Spread of Vancomycin Resistance have discouraged the use of oral vancomycin for the treatment of CDAD except for failures of metronidazole or severe potentially life-threatening illness.<sup>174</sup>

Other regimens that have been compared in randomized therapeutic trials for CDAD include teicoplanin, bacitracin, and colestipol (Table 5). Treatment with teicoplanin at 100 mg twice daily for 10 days achieved response rates similar to those achieved with vancomycin.<sup>175</sup> Clinical cure rates and *C difficile* eradication rates with bacitracin are somewhat lower than with vancomycin,<sup>176,177</sup> and bacitracin should be considered as a second-line agent in the treatment of CDAD. Treatment with the ion-exchange resin, colestipol,

clearly is inferior to the agents described above<sup>178</sup> and is not recommended for initial treatment of CDAD.

### Treatment of Complicated Infections

Recurrences of CDAD with the original (ie, relapse) or a new infecting strain occur in 5% to 30% of patients successfully treated with any regimen, and some patients have multiple relapses.<sup>104,143</sup> The mechanism of diarrhea relapse may be different following treatment with metronidazole and vancomycin.<sup>179</sup> Fecal levels of metronidazole fall rapidly with resolution of diarrhea,<sup>165</sup> permitting germination of any remaining *C difficile* spores. Vancomycin, unlike metronidazole, exerts a bacteriostatic effect on *C difficile* at the high concentrations achieved during therapy, suggesting that a large portion of organisms may remain viable during therapy.<sup>179</sup>

Regardless of the mechanism of diarrhea recurrence, patients will respond again to the same specific therapy, and 92% will not experience further recurrences.<sup>143</sup> For those patients who experience two or more recurrences, a number of empiric management strategies may be employed, the rationale of these strategies being an attempt to reestablish the normal colonic flora: treatment with vancomycin followed by *Saccharomyces boulardii*<sup>80</sup>; combined treatment with vancomycin and rifampin<sup>181</sup>; metronidazole or bacitracin followed by *Lactobacillus GG*<sup>182</sup>; vancomycin followed by cholestyramine<sup>183,184</sup>; vancomycin followed by synthetic fecal bacterial enema<sup>185</sup>; administration of a nontoxigenic *C difficile* strain<sup>186</sup>; treatment with vancomycin in tapering doses<sup>187</sup>; or no treatment with careful observation.<sup>163</sup> Use of combined vancomycin and rifampin is among the simplest approaches for treatment of multiple recurrences. A blinded, controlled trial showed that the addition of 4 weeks of *S boulardii* to standard antimicrobial therapy in the

TABLE 6  
SUMMARY OF RECOMMENDATIONS WEIGHTED FOR STRENGTH OF RECOMMENDATION AND QUALITY OF EVIDENCE TO SUPPORT THE RECOMMENDATION<sup>194</sup>

|   | Strength of Recommendation* | Quality of Evidence† | References  |
|---|-----------------------------|----------------------|-------------|
| <b>A. Diagnosis</b>   |                             |                      |             |
| 1. It is recommended that tests for <i>C difficile</i> or its toxins be performed only on diarrheal (unformed) stool specimens unless ileus due to <i>C difficile</i> is suspected.   | B                           | III                  | 9-11        |
| 2. Testing of stools of asymptomatic patients for <i>C difficile</i> or its toxins is not clinically useful (including "tests of cure") and is not recommended except for epidemiologic investigation purposes.   | B                           | III                  | 10,169,195  |
| 3. Clinical illness usually does not correlate with the presence of <i>C difficile</i> or its toxins in the stools of infants under 1 year old; testing of these patients is discouraged.   | B                           | III                  | 20,75,78    |
| 4. Stool culture is the most sensitive test for <i>C difficile</i> -associated diarrhea (CDAD), whereas stool cell cytotoxicity (toxin B) is the most specific; for maximal diagnostic sensitivity and specificity, performance of both tests is recommended. | A                           | II                   | 12,13,31-33 |
| 5. Enzyme immunoassays for toxin A are rapid but may be less sensitive or less specific than cell cytotoxin assays; use of enzyme immunoassay in place of cytotoxin assay is recommended as an acceptable alternative to the cell cytotoxin assay.            | B                           | II                   | 28,32,36-42 |
| 6. The latex agglutination test detects glutamate dehydrogenase and is not as sensitive as culture, cell cytotoxin, or enzyme immunoassay tests; its use is discouraged.  | A                           | II                   | 12,31,38,57 |
| <b>B. Epidemiology</b>  |                             |                      |             |
| 1. <i>C difficile</i> is the most frequently identified cause of nosocomial diarrhea; stool testing for <i>C difficile</i> and its toxins is recommended in hospitalized patients who have diarrhea.  | A                           | II                   | 4,5         |
| 2. Antimicrobial (or antineoplastic) treatment is a nearly universal risk for CDAD; obtaining a history for administration of these agents within the prior 2 months is recommended before <i>C difficile</i> diagnostic studies are performed.               | A                           | II                   | 14,89,91,93 |
| 3. In the investigation of nosocomial CDAD, it is recommended that exogenous acquisition be of primary concern; disease due to endogenous organisms is rare except in recurrent CDAD.   | A                           | II                   | 6,7,106     |
| 4. It is recommended that when the CDAD incidence is high, that the rate of asymptomatic <i>C difficile</i> stool colonization also should be assumed to be high and to increase with duration of hospitalization.  | A                           | II                   | 6,7,93,106  |
| <b>C. Prevention and control</b>  |                             |                      |             |
| 1. Personnel glove use for the handling of body substances of all patients is recommended to reduce the rate of CDAD.   | A                           | I                    | 110         |
| 2. Handwashing with either an antimicrobial agent or soap is recommended after contact with patients, their body substances, or environmental surfaces.   | B                           | III                  | 138         |
| 3. Replacement of electronic thermometers with disposable thermometers is recommended if CDAD rates are high.   | A                           | II                   | 113         |
| 4. Antimicrobial use restriction is indicated if a specific antimicrobial, particularly clindamycin, is identified as a risk for CDAD in the institution.   | A                           | II                   | 88,140      |
| 5. Isolation of patients with CDAD in private rooms is recommended if private rooms are available; priority should be given to patients unable to maintain bowel continence and good handwashing hygiene.   | B                           | III                  | 143         |

(Table continued on page 473)

TABLE 6 (continued)

SUMMARY OF RECOMMENDATIONS WEIGHTED FOR STRENGTH OF RECOMMENDATION AND QUALITY OF EVIDENCE TO SUPPORT THE RECOMMENDATION<sup>194</sup>

|  | Strength of Recommendation* | Quality of Evidence† | References  |
|--|-----------------------------|----------------------|-------------|
| D. Treatment   |                             |                      |             |
| 1. If clinically appropriate, discontinuation of offending antimicrobials is recommended if CDAD is suspected; 20% to 25% of patients will respond to discontinuation without further treatment.             | A                           | I                    | 162         |
| 2. Metronidazole or vancomycin for 10 days are recommended effective treatments; metronidazole is less expensive and may be preferable to avoid vancomycin resistance in other nosocomial bacterial species. | B                           | I                    | 143,162,174 |
| 3. Treatment of asymptomatic patients colonized with <i>C difficile</i> is not recommended.  | A                           | I                    | 152         |
| 4. For patients who have a first recurrence of diarrhea following treatment of CDAD, m-treatment in the same manner as for the initial episode (metronidazole or vancomycin) is recommended.                 | B                           | III                  | 143         |

\* Categories for strength of recommendation: A, good evidence for support; B, moderate evidence for support; C, poor evidence to support.

† Categories reflecting the quality of evidence on which recommendations are based: I, evidence from at least one properly randomized controlled trial; II, evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one center), from multiple time-series studies, or from dramatic results in uncontrolled experiments; III, evidence from opinions of respected authorities, based on clinical experience, descriptive studies, reports, or expert committees.

treatment of recurrent (but not initial) CDAD significantly decreased the rate of recurrence when compared to antimicrobial treatment alone.<sup>188</sup> In children with chronic relapsing CDAD and low levels of serum IgG antibodies to *C difficile* toxin A, treatment with intravenous gamma globulin resulted in clinical and bacteriological improvement.<sup>189</sup> Some of these approaches are less practical than others, most are anecdotally reported, and review of the primary reference before employing one of these strategies is advised. The best approach for management of repeated CDAD recurrences is unknown at present, but it is reassuring that over 90% of patients respond simply to being retreated with vancomycin or metronidazole after the first recurrence and that with commercial availability of an *S bolearii* preparation in the United States, the response rate is likely to improve further.<sup>143,188</sup>

The most difficult and controversial issues in the treatment of *C difficile* infections are in the management of patients with toxic megacolon or ileus. It is important to remember that these patients may have atypical symptoms presenting without diarrhea and/or mimicking an acute surgical abdomen.<sup>143,190</sup> Delayed recognition or failure to consider *C difficile* as the cause in this setting can lead to severe complications. Management of these patients, again, has been empiric, but one goal is to achieve effective antimicrobial concentrations at the site of infection when the oral route is compromised. Some authors advocate

treatment with intravenous metronidazole or with intravenous vancomycin at dosages  $\geq 2$  g/day, placement of a long catheter in the small intestine and instillation of vancomycin, or instillation of vancomycin by enema.<sup>191</sup> Another approach used successfully in six of eight patients with severe ileus at one institution employed vancomycin administered by nasogastric tube and by retention enema plus intravenous metronidazole.<sup>143</sup> Surgical intervention is indicated in patients with toxic megacolon who are not responding to medical treatment or when colonic perforation is suspected.<sup>190</sup> Colonic diversions and partial or complete colectomies have been performed, but mortality is high.<sup>190</sup>

## SUMMARY

Despite the considerable body of data assembled in the literature and reviewed in this paper, it is clear that CDAD persists and likely is increasing in frequency despite the best efforts to control and prevent it in institutions.<sup>143,192,193</sup> Summary recommendations and conclusions are presented in Table 6 together with a determination of the strength of the recommendations and quality of the supporting evidence.<sup>194</sup> New and innovative approaches are needed in every aspect of *C difficile* study: diagnosis, epidemiology, pathophysiology, immunity, prevention, control, and treatment. We hope this position paper will be a stimulus for carefully planned, well-controlled, prospective future studies of this persistently important illness.

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