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Number XXX

Effectiveness of Screening and Treatment of C difficile Infections

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Preface

The Agency for Healthcare Research and Quality (AHRQ) conducts the Effective Health Care Program as part of its mission to organize knowledge and make it available to inform decisions about health care. As part of the Medicare Prescription Drug, Improvement, and Modernization Act of 2003, Congress directed AHRQ to conduct and support research on the comparative outcomes, clinical effectiveness, and appropriateness of pharmaceuticals, devices, and health care services to meet the needs of Medicare, Medicaid, and the Children's Health Insurance Program (CHIP).

AHRQ has an established network of Evidence-based Practice Centers (EPCs) that produce Evidence Reports/Technology Assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care. The EPCs now lend their expertise to the Effective Health Care Program by conducting Comparative Effectiveness Reviews (CERs) of medications, devices, and other relevant interventions, including strategies for how these items and services can best be organized, managed, and delivered.

Systematic reviews are the building blocks underlying evidence-based practice; they focus attention on the strength and limits of evidence from research studies about the effectiveness and safety of a clinical intervention. In the context of developing recommendations for practice, systematic reviews are useful because they define the strengths and limits of the evidence, clarifying whether assertions about the value of the intervention are based on strong evidence from clinical studies. For more information about systematic reviews, see http://effectivehealthcare.ahrq.gov/reference/purpose.cfm.

AHRQ expects that CERs will be helpful to health plans, providers, purchasers, government programs, and the health care system as a whole. In addition, AHRQ is committed to presenting information in different formats so that consumers who make decisions about their own and their family's health can benefit from the evidence.

Transparency and stakeholder input from are essential to the Effective Health Care Program. Please visit the Web site (www.effectivehealthcare.ahrq.gov) to see draft research questions and reports or to join an e-mail list to learn about new program products and opportunities for input. Comparative Effectiveness Reviews will be updated regularly.

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Structured Abstract

Objectives: To conduct a systematic review and synthesize evidence for the effects of diagnostic tests, prevention, and treatment of C. difficile associated disease (CDAD) in adult patients.

Data Sources: Searching for relevant literature was conducted in MEDLINE®, the Cochrane Library, and Allied and Complementary Medicine (AMED). ClinicalTrials.gov and expert consultants provided leads to additional studies. We also manually searched reference lists from relevant systematic reviews.

Review Methods: Standard Evidence-based Practice Center methods were employed. Screening of abstracts and full text articles to identify studies meeting inclusion/exclusion criteria was performed by two independent reviewers. High quality experimental design studies were used to examine differences in comparative diagnostic tests. Randomized controlled trials were used to examine comparative effectiveness of antibiotic treatment for CDAD. Data extraction was completed and quality was checked by separate reviewers. Quality ratings and strength of evidence grading was performed on included studies. Evidence on diagnostic tests was quantitatively synthesized focusing on differences between test sensitivities and specificities. Evidence on antibiotic treatment was quantitatively examined using pooled analysis. Qualitative narrative analysis was used to synthesize evidence from all available study types for environmental prevention and nonstandard prevention and treatment, with the exception of probiotics as primary prevention, for which a Forest plot is provided.

Results: Overall, literature was sparse and strength of evidence was low due to small sample sizes or lack of adequate controls. For diagnostic testing, commercially available enzyme immunoassays do not differ in sensitivity or specificity. Gene-based testing using PCR appears to be more sensitive without loss of specificity. Limited evidence suggests that a two-step gene-based method may enhance specificity but reduce sensitivity. Moderate evidence in favor of antibiotic restriction policies for prevention was found. Environmental preventive interventions such as glove use and disposable thermometers have limited evidence to support their practice. However, this literature is largely based on controlling outbreaks. Use of multiple component interventions further limits the ability to synthesize evidence in a meaningful way. Numerous potential new forms of treatment are being examined in placebo controlled RCTs, case series, and case reports. For standard treatment, no differences were found between vancomycin and metronidazole in a total sample of 330 patients except possibly severe cases, although this is based on only 69 patients and severity is not well defined in the literature. Monoclonal antibodies for prevention and bacteriotherapy for multiple recurrences appear promising.

Conclusions: Given the frequency and severity of CDAD and the fact that future reimbursement policy may withhold payment for hospital-acquired infections, this is an under-researched topic. Data and practices are not in place to definitively determine that infection manifesting in the hospital was hospital-acquired. More research on effective treatment and unintended consequences of treatment, such as resistance, is needed. Gut flora may be important, but improved understanding of healthy gut ecology and the complex interactions is necessary before continuing to pursue probiotics.

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Executive Summary

Introduction

C. difficile-associated disease (CDAD) is an important hospital-acquired infection and a growing health care problem. *C. difficile* is a gram-positive, spore-forming, anaerobic bacterium that, when ingested, can cause CDAD if it is a toxigenic strain. CDAD symptoms can range from mild diarrhea to severe cases, including pseudo membranous colitis and toxic megacolon. CDAD incidence is estimated at 6.5 cases per 10,000 patient days in hospital.[1] About 250,000 hospitalizations were associated with CDAD in 2005.[2] Mortality from CDAD is estimated at 7 percent of cases.[3] The vast majority of severe morbidity and mortality is experienced by elderly people in hospitals.[4-6] Residents of long-term care facilities (LTC) are also at higher risk, with up to 26.2 cases per 10,000 resident days versus 6.5 cases per 10,000 hospital days.[7] Incidence rates may increase by four or five fold during outbreaks.[8] In addition to institutional care environments, *C. difficile* is also common in the community, being easily isolated from soil and water samples.[9] Community associated CDAD rates are generally much lower, accounting for 27 percent of all CDAD cases in a recent prevalence study[8] but are also on the rise.[10] However, the source of the *C. difficile* organisms responsible for cases of CDAD in the community is not well understood.

In order for CDAD to develop, a person must be infected with a strain of *C. difficile* capable of making toxin in the person's colon. Toxigenic strains include those that make toxin A (a cytotoxin), toxin B (an enterotoxin), or both toxins. Infection with both toxigenic and nontoxigenic strains commonly occurs in healthy individuals. If these persons have usual, healthy colonic flora, the risk of CDAD is exceedingly low. There is a small risk of CDAD if the colon flora becomes disturbed, commonly through antibiotic use, while the person is infected (colonized) with a toxigenic strain. Antibiotics that disturb colon flora enough to allow CDAD to develop must get into the colon, and they are associated with profound alterations in relative amounts of colon bacterial constituents.[11,12] The immune status of the patient also contributes to the risk of developing CDAD and the experienced severity.[13] Other risk factors include increasing age, female gender, comorbidities, gastrointestinal procedures, and use of gastric acid suppressant medications (although the last is still controversial).[14-18] Risk profiles for recurrent CDAD are similar.[15] One study, which statistically modeled CDAD within the hospital setting, suggested that reducing patient susceptibility to infection is more effective in reducing CDAD cases than lowering transmission rates.[19]

New, more virulent strains have emerged since 2000. Characteristics associated with hypervirulent strains include increased toxin production (due to a deletion in a toxin regulatory gene), an additional binary toxin, whose role in disease etiology is not well understood, hypersporulation, and high-level resistance to fluoroquinolone antibiotics.[20] These new strains affect a wider population, often people with a lack of established risk factors for CDAD based on older strains, such as previous hospitalization or antibiotic use, and include children, pregnant women, and other healthy adults.[21] With hypervirulent strains the time from symptom development to septic shock may be reduced, making quick diagnosis and proactive treatment regimens critical for positive outcomes.

The highly virulent strain associated with the epidemic of CDAD described in the early 2000s may be decreasing in prevalence in limited locations.[22] Recent analysis of an archived

collection of *C. difficile* isolates revealed that predominant strains shifted from year to year among a population served at a single institution,[23] suggesting that this strain shift may occur on a larger scale. However, this phenomenon potentially cuts both ways as strains drift toward lesser or higher virulence, and the possible future risks and costs of CDAD remain significant.

Scope and Key Questions

The purpose of this systematic review was to provide an overarching assessment of the evidence for comparing the accuracy of diagnostic tests and the effectiveness of prevention and treatment interventions on initial and recurrent CDAD related patient outcomes in adult patients. This purpose was developed during the project's topic refinement stage. There was consensus among key informants that the single greatest contribution this systematic review could contribute to the field was to provide a comprehensive review by an independent organization that covered the major concerns of the field. CDAD is an active topic in the literature as well as a vital clinical concern. The consensus opinion included the idea that clinicians and researchers both would be well served to reaffirm what is and is not supported by evidence in the literature, and at what level of evidence, to balance against this activity level.

The general use of the term *C. difficile* associated disease, or CDAD, rather than *C. difficile* infection (CDI) underlines the major impetus of this review, which is concern for the presence of clinical disease, not asymptomatic carriage of the *C. difficile* organism. While we were interested in how treatment of CDAD varies by organism strain, molecular epidemiology studies whose main purpose was to identify the strains of *C. difficile* present in the population are also outside the scope of this review. The review focuses on adult patients because adults, and particularly elderly adults, carry the large majority of the morbidity and mortality burden.

The following key questions form the basis for this review:

Key Question 1. How do different methods for detection of toxigenic *C. difficile* to assist with diagnosis of CDAD compare in their sensitivity and specificity?
(a) Do the differences in performance measures vary with sample characteristics?

Key Question 2. What are effective prevention strategies?
(a) What is the effectiveness of current prevention strategies?
(b) What are the harms associated with prevention strategies?
(c) How sustainable are prevention practices in health care (outpatient, hospital inpatient, extended care) and community settings?

Key Question 3. What are the comparative effectiveness and harms of different antibiotic treatments?
(a) Does effectiveness vary by disease severity or strain?
(b) Does effectiveness vary by patient characteristics: age, gender, comorbidity, hospital versus community acquired setting?
(c) How do prevention and treatment of CDAD affect resistance of other pathogens?

Key Question 4. What are the effectiveness and harms of nonantibiotic adjunctive interventions?
(a) In patients with relapse/recurrent CDAD?

Methods

We used the key word "*difficile*" to identify all articles related to *C. difficile*. Articles were limited to English language and humans. No date limits were applied. We searched MEDLINE®, AMED, the Cochrane library, and ClinicalTrials.gov. For systematic reviews, we searched MEDLINE®, the Cochrane Database of Systematic Reviews, and the Web sites of the National Institute for Clinical Excellence, Guidelines.gov, and the NHA Health Technology Assessment Programme. We also manually searched reference lists of review articles and articles that were read for the review.

For Key Question 1, we included studies that used clinical stool specimens from patients suspected to have CDAD. We included studies that concurrently compared at least two diagnostic tests in the same laboratory using the same stool samples from cases of clinically suspected CDI using the same reference standard to reduce heterogeneity in the estimates. Studies must have used toxigenic culture, cell cytotoxicity assay, or combinations of tests, as the reference test for toxigenic CDI. Direct comparisons of diagnostic tests without a reference test were not included. We present study results in positive terms, i.e., true positives (sensitivity) and false positives (1-specificity).

For Key Question 2, we included studies that examined the effects of prevention strategies aimed at breaking routes of transmission within institutional settings or reducing susceptibility to CDAD through antibiotic prescribing practices. We included only studies with CDAD incidence, or other measures of CDAD, as an outcome. We excluded studies that used only intermediate outcomes, such as reduced spore count in environmental samples. Accepted study designs included randomized controlled trials (RCT), prospective cohort, retrospective cohort, time series, and before/after trials. We also identified good quality studies that identified specific risk factors for development of CDAD to facilitate infectious disease control efforts to target likely effective preventive strategies.

For Key Question 3, we included RCTs that compared two active treatments, vancomycin, metronidazole, bacitracin, nitazoxanide, rifaximin, and rifampin, on adult inpatients. We also included placebo controlled trials for vancomycin or metronidazole, the agents of most interest. We included initial cure, recurrence (variably defined by symptoms with or without a positive test for *C. difficile*), and mortality, which are outcomes of interest to clinicians and are reported in most studies. We also included time to resolution of diarrhea,

Diagnostics (KQ1) Results

We found ten references that provided comparative data about diagnostic tests of interest.[24-33] The number and type of paired (within study) comparisons available for each diagnostic test varied considerably, and not all possible comparisons were available.

Fifteen paired comparisons of seven commonly used immunoassays for toxins A and B provided low grade evidence that the test sensitivities do not differ. There was moderate grade evidence for no differences in test specificities for two comparisons and for a difference of 2 percent in one comparison. Otherwise, there was only low grade evidence for or against differences in test specificities. There was insufficient evidence of differences between all tests that were not directly compared.

Nine comparisons of two different gene detection tests to toxin immunoassays or a 2-stage that first tested for GDH to provided only low grade evidence that the gene-based tests are substantially more sensitive. There was moderate evidence that the test specificities in one comparison did not differ. Otherwise, there was only low grade evidence for differences in either direction between test specificities. There was insufficient evidence of differences between all tests that were not directly compared.

There was only low grade evidence that combining an immunoassay for GDH followed by an immunoassay of GDH positive specimens for toxins A and B reduced the percentage of false positives (increased the test specificity), but reduced the test sensitivity. There was insufficient evidence of differences between all tests that were not directly compared.

There was insufficient evidence to determine whether any differences in sensitivity or specificity between diagnostic tests depend on patient or specimen characteristics or the clinical scenarios that lead to testing for toxigenic CDI.

Prevention (KQ2) Results

We found one Cochrane review, [34] four studies on antibiotic prescribing restrictions,[35-38], 10 on single preventive practices aimed at transmission interruption,[39-48] and 10 studies that bundled multiple practices into a prevention strategy.[49-58] Twelve studies examining risk factors met the inclusion criteria and updated the period following a systematic review.[14]

Overall, the evidence available to link prevention strategies to clinically important outcomes, such as CDAD incidence, is of low quality, and is not extensive.

Four observational studies[35-38] and one Cochrane Review,[34] found prescribing practice interventions decreasing the use of high risk antimicrobials are associated with decreased CDAD incidence. Prescribing practices were also used in multicomponent interventions credited with reducing CDAD incidence; however, it is difficult to isolate the specific effects of the prescribing practices.

One controlled trial found glove use significantly reduced CDAD incidence in the hospital setting.[39] Likewise, three observational studies, including two controlled, found disposable thermometer use is likely to reduce CDAD incidence.[40-42]

No study examined the effect of handwashing, rather than alcohol gels, on CDAD incidence. Four studies found use of alcohol gels, presumably in the presence of protocols requiring handwashing in the presence of CDAD or visible soiling, did not increase CDAD incidence.[43-45,59]

Three single component intervention studies provide low evidence that disinfection with a chemical compound that kills *C. difficile spores* in the hospital environment prevents CDAD, at least in epidemic or hyperendemic settings.[46-48] Seven studies included disinfection in multicomponent interventions.[50,52,53,56,60] Disinfection agents examined included hypochlorite solution, hydrogen peroxide, aldehydes, and detergent.

Ten time series/before-after studies have examined bundled multiple interventions using before/after study designs.[49-58,60] All of the studies described the use of the measures to control epidemic CDAD, or endemic CDAD which was felt to be excessive. The number of interventions, and the specific nature of any particular intervention, varied widely. Studies employed between two and nine different types of interventions. Data is insufficient to draw conclusions.

Risk factors for developing CDAD include antibiotic use, substantial chronic illness, hospitalization in an ICU, and age. There are conflicting data about drugs used for acid suppression.

No data on patient harms or harms to hospital staff due to preventive interventions were reported. Likewise, no studies assessed the sustainability of a prevention program beyond an intervention period.

Standard Treatment (KQ3) Results

Ten randomized clinical trials were identified that evaluated different antimicrobials (or different doses of a single drug) available for treatment of CDAD in the United States.[61-70] These ten studies, published from 1978 to 2009, ranged in size from 39 to 172 subjects.

Overall, study quality is low. Vancomycin and metronidazole, the most frequently clinically used antimicrobials, were also the most frequently compared antimicrobials. Three RCT comparisons of vancomycin to metronidazol, with a total of 335 pooled subjects, found no significant differences in any examined outcome.[62,65,68] One RCT comparing vancomycin to metronidazole, using a pre-specified subgroup analysis of 69 patients, found a small but significant increase in the proportion of subjects with severe CDAD who achieved initial clinical cure with vancomycin.[62]

No other head to head trial demonstrated superiority of any single antimicrobial for initial clinical cure, clinical recurrence, or mean days to resolution of diarrhea. Combination therapy with rifampin and metronidazole resulted in significantly higher mortality when compared to treatment with metronidazole only.[63] Pooled data of 104 subjects comparing vancomycin to bacitracin showed significantly higher rates of organism or toxin clearance for vancomycin.[66,69]

Harms were not reported with sufficient detail to compare the risks of any particular antimicrobial with another antimicrobial. When harms were reported, they were generally nonserious (e.g. nausea, emesis) and transient.

No data were available to assess the importance of general patient characteristics or the strain of organism on the effectiveness of an antimicrobial.

Non-Standard Treatment (KQ4) Results

Six RCTs on nonantibiotic adjunctive treatments of CDAD, and 12 studies addressed prevention of CDAD, formed the basis of this analysis. Five of the studies on treatment of CDAD compared a nonantibiotic intervention with an active control, i.e., a standard antibiotic treatment for CDAD, oral vancomycin or metronidazole.[71-75] One study compared a nonantibiotic intervention with placebo.[76] All but one[73]of the 12 prevention studies compared the nonantibiotic intervention to placebo rather than to another intervention, reflecting the current state of this area of science. Numerous published case reports, as well as nonexperimental studies, describe additional nonantibiotic approaches for treatment of CDAD and their possible harms. As found with the other key questions, overall, study quality was low. Definitions of CDAD with regard to diarrhea, i.e., number and consistency of stool, were inconsistent across studies.

For treatment of CDAD, *C. difficile* immune whey[74] to enhance immune response, and colestipol as a toxin binding agent,[76] are not more effective in treating CDAD than standard antibiotic treatment with oral vancomycin, metronidazole, or placebo. Probiotics administered as

an adjunct to antibiotic treatment were not more effective than treatment with antibiotics alone.[71,72,75]

There is low quality limited evidence that the probiotic[77-82] and toxin binding Tolevamer[73] interventions in this review are not more effective than placebo for primary prevention of CDAD. There is low quality limited evidence from one subgroup analysis that a prebiotic may reduce diarrhea recurrence in patients treated for CDAD more so than placebo with standard antibiotics.[83,84] There is also limited moderate quality evidence from one study that monoclonal antibodies are effective in preventing recurrence of CDAD.[85]

There is limited low quality evidence from two case series that fecal biomass transplant is effective in treating recurrent CDAD for up to 1 year.[86,87]

Discussion

There is very limited high quality evidence, to support the diagnostic, preventive, and treatment practices for CDAD carried out by providers in hospital, long-term care, and outpatient settings. Table 22 provides a summary of the evidence and results presented in this review. Inconsistency in definitions of diarrhea, severity, resolution of symptoms, recurrence, or cure contributes to the difficulty in drawing conclusions from the evidence.

In general, there's little evidence that the sensitivities of commonly used immunoassays for toxins A and B differ, and any differences in their percent of false positives (1 – specificity) most likely are small (3 percent or less). However, the strength of the evidence is low due to the number of immunoassay comparisons that are available in the literature. The possibility exists that future research could impact the findings. Further, one article with eight comparisons is the sole source for many of the comparisons. The available comparative data doesn't rule out the possibility of larger differences between some of the immunoassays that have or have not been directly compared in adequate numbers. While the precision of the findings is such that we cannot rule out the possibility of differences in sensitivity on the order of three to five percent, it is unclear whether such differences would affect clinical decisionmaking.

Gene-based tests that used toxin B gene fragments tended to have better sensitivity than immunoassays for toxins A and B. Results, however, should be viewed with caution. Few studies contributed to the findings, and many direct comparisons were not found. Further, variation in the stability of the toxins in stool specimens as they were collected, stored, and processed, may have contributed to the observed variation between studies in the estimates of the immunoassays sensitivities, whereas detection via amplification of gene fragments could be less susceptible to specimen degradation.

These tests require varying skills, equipment, and time to carry out, and heterogeneity is a significant factor in reviewing the literature. Previous reviews by Planche et al.[88] and Crobach et al.[89] encountered difficulty comparing the sensitivities and specificities of the diagnostic tests in large part because there was too much variation between studies in the estimates of sensitivity and specificity of a particular test. We attempted to control for the heterogeneity between studies by examining the differences in sensitivity and specificity in stool samples tested within the same lab and did not find strong evidence of differences between tests within several immunoassays for toxins type A and B. The extent of any publication bias for these comparisons is unknown.

A clinically important question is whether the potential differences in the accuracy of the diagnostic tests being employed in practice would translate into differences in clinical behaviors or patient outcomes. Indeed, how well clinicians actually know the sensitivity and specificity of

the test(s) for toxigenic CDI employed by their laboratories and incorporate this information into their patient care decisions is not clear.

Very little evidence connects prevention strategies and techniques directly to patient related outcomes, such as CDAD incidence. Available evidence is generally from before/after study designs or limited time series. Hospital settings with outbreaks or hyperendemic episodes further limit applicability of the findings, and leave open the question of the relative contribution of regression to the mean (i.e., that CDAD rates returned to baseline rates even in the absence of effective interventions). The studies also varied in the degree to which they described CDAD surveillance, diagnostic accuracy, or laboratory performance. In most, surveillance was passive and depended on a positive toxin test on a stool specimen sent by clinicians caring for a patient with diarrhea. Unknown numbers of cases might have been missed or misdiagnosed. Additionally, attention has not been given to describing a prevention strategy's potential harm (e.g., increase in other pathogens, reduction in direct patient care contact due to isolation or restrictive contact requirements, increased costs) or the long-term sustainability of a practice.

There is low quality evidence that antibiotic prescribing practices appear to reduce CDAD incidence, a finding consistent with the Cochrane review.[34] None of the studies explicitly addressed the potential harms of changes in antibiotic use policy, but there are several theoretical harms. They include the possibility that preferred drugs will be less effective than drugs that physicians are discouraged from using, or drugs that are made unavailable for treating infections other than CDAD. Preferred antimicrobials might have greater costs or greater toxicities unrelated to CDAD. *C. difficile* strains might evolve to develop resistance to the preferred antibiotics, which might increase the likelihood that the recommended antibiotics might induce CDAD.

While several studies found increased risk with specific antibiotics or antibiotic classes, the antibiotics that confer greater risk for CDAD have changed over time and vary by location because of differences in prevalent toxigenic strains and especially the susceptibility patterns of those strains.[90] Clindamycin resistance was identified soon after the role of *C. difficile* in pathogenesis was discovered.[39,91,92] More recently, quinolones have assumed greater importance because strains have become more resistant over time.[93]

Fewer studies are available to support prevention practices aimed at breaking transmission. There was limited low quality evidence that gloves, disposable thermometers, handwashing, and intensive disinfection solutions help to reduce CDAD incidence. In addition, the presence and use of alcohol gel to prevent other hospital acquired infections, such as MRSA, did not increase the rate of CDAD incidence, suggesting the possibility that providers and hospital staff did not substitute alcohol gel for hand washing.

Similar to the antibiotic prescribing practice research, none of the studies aimed at breaking transmission addressed potential harms for other prevention practices. Costs of disinfection, time to perform disinfection, and the possible harm to surfaces and equipment should be anticipated. Failures with vapor disinfection systems would be possible and might lead to toxic exposures of personnel or patients. Nor is there evidence to inform infection control professionals whether such practices are sustainable after an intervention period. That is, we cannot answer whether environmental cleaning staff will have developed professional habits that will continue when the intense monitoring related to an intervention period discontinues.

The potential for prevention research is often compromised by the swift uptake of newly described prevention strategies with the belief that these will improve institutional practices, health care quality, and reduce CDAD morbidity and mortality. Current prevention strategies

often rely on studies using intermediate outcomes such as process. Newly acquired strategies are then added to current practice, bundling them into multiple component interventions. Multiple component prevention strategies document the multipronged prevention efforts in natural settings and have been reported in the literature to be successful in response to outbreak or hyper-endemic situations. It is very difficult, if not often impossible, to tease out the relative contributions of single components to the overall multiple component bundle of prevention strategies.

The available evidence is insufficient to say whether any antimicrobial treatment is better than another, including the two most commonly used treatments, metronidazole and vancomycin. The total number of subjects from comparative studies on metronidazole and vancomycin is just 335 patients. This raises the possibility that, although a significant difference in effectiveness has not been detected, a true difference may exist. There is also no evidence for a difference in effectiveness for other agents, but again the possibility remains that such a difference exists. However, at this time any claims that one agent is superior to another for all cases of CDAD are not supported by available evidence. The findings apply to general adult inpatients. Bias due to selectively reporting outcomes is possible if cut-points are changed for CDAD definitions, e.g., number or consistency of stools. The clinical differences of changes in cut-points are also unknown, however, so the clinical significance could remain.

We found limited low-quality evidence that vancomycin was superior to metronidazole for subjects classified as having severe disease. This finding came from a sub-group analysis of a single trial; thus, confirmation is needed. Strengthening this finding is the fact that it was a pre-specified analysis, and that the severity classification appears to have been made before treatment allocation. An argument can be made that even small increases in effect size are important to a high risk patient population where time is of the essence.[94]

We sought to document the range of treatments under investigation for treatment and prevention of CDAD, particularly recurrent CDAD. The evidence for effectiveness of nonantibiotic interventions for treating CDAD shows that probiotics, prebiotics, tolevamer, *C.* difficile immune whey, and colestipol are not more effective in treating CDAD than standard antibiotic treatment with oral vancomycin or metronidazole or compared with placebo. The evidence supporting this conclusion is limited and of low quality.

Prevention of CDAD, both initial and recurrent cases, through interventions intended to improve gut flora and host immunity is also a very active topic in the literature. There is limited, low quality evidence that the nonantibiotic prevention interventions are not more effective than placebo for primary prevention of CDAD. There is limited evidence of low quality that administering the prebiotic oligofructose or a monoclonal antibody to *C. difficile* toxins A and B along with standard antibiotics for CDAD are better than placebo and active control in preventing recurrence of CDAD in patients treated for CDAD. Although the studies for both treatment and prevention of CDAD using a nonantibiotic intervention included components of experimental designs, few had adequate rigor to yield high quality findings or power to detect a significant difference between the interventions (or placebo) compared. In some studies, a low rate of CDAD precluded statistical testing.

Caution is recommended regarding new, nonstandard treatments, and not extrapolating study findings beyond the data. For example, one cannot assume if a probiotic treatment is effective for Antibiotic Associated Diarrhea that it will be effective for CDAD. Likewise, attention should be paid to which patients were included and excluded in probiotic treatment studies. Such studies generally exclude high-risk patients. Thus, there is no evidence for the use of probiotics in high

risk patients. During a TEP call it was noted that some ICUs have banned probiotics in order to avoid the potential for fungemia and bacteremia in very ill patients.

Future Research

There are a number of important questions to be addressed regarding diagnostic testing, prevention, and treatment of CDAD. Executive Summary Table 2 provides a summary of the research recommendations.

Diagnostic tests. It is difficult to apply the available evidence from comparative studies to help select the best diagnostic test(s) for clinical applications. The reviewed comparative studies did not clearly define the testing scenario including the setting, disease prevalence, patient selection criteria, patient characteristics, or the signs and symptoms of the suspected CDAD, making it difficult to judge to whom the study results might apply. Ultimately, the clinical importance of estimated differences in sensitivity (true positives), false positives, specificity (true negatives) and false negatives, depends on how these types of test results would affect clinical decisions and patient outcomes.

More research is needed to understand how test sensitivities and specificities are used to make decisions in clinical practice, and to define clinically meaningful differences based on their effects on clinical decisions and patient outcomes. Multicenter studies that (1) consistently use the most clinically relevant reference test, (2) use explicit clinical criteria to select patients and stool specimens to be tested, (3) randomly assign patients to different diagnostic tests, and (5) use key clinical outcomes as study endpoints are needed to fill this major gap in knowledge about diagnostic tests for toxigenic *C. difficile*.

Whether the newer gene amplification and detection tests are more consistent across laboratories, and more sensitive than the currently used toxin immunoassays without substantial loss of specificity, needs further study. Most importantly, studies are needed to demonstrate that use of tests that detect genetic residue related to *C. difficile* toxin production rather than the toxins *per se* lead to better patient outcomes.

Prevention. A number of potential prevention strategies can, and should, be investigated as a single intervention in a controlled trial in order to understand its potential contribution to a prevention program. However, the main obstacle to research in this area is the contextual setting.

Prevention happens within an institutional environment, as a comprehensive approach for multiple potential hospital acquired infectious agents, and attending to multiple potential vectors of transmission and host susceptibility. Researchers and decisionmakers may need to consider another approach to inform decisionmaking: a collaborative research process in which consensus agreements are reached for minimum data sets and followup periods, and definitions of interventions are agreed to in order to facilitate pooling data across organizations. Datasets of this nature could allow for employing more sophisticated epidemiological and decision analytic techniques to tease apart the relative contributions of different prevention strategies. The nature of the decisions faced by infection control professionals is qualitatively different than a physician's clinical decisions for an individual CDAD patient. Decision analytic techniques may be particularly valuable in this venue.

Standard treatment. The greatest needs for future studies for CDAD treatment are consistent definitions and reporting of outcomes, a uniform and clinically relevant definition of disease severity, and trials with adequate power to detect clinically meaningful differences in outcomes. In particular, trials need to include adequate numbers of subjects to allow stratification by patient characteristics such as age, gender, and comorbid conditions in order to address

questions regarding the most effective therapy for CDAD. A well validated and clinically meaningful severity score would also assist in treatment decisions. Although most agents for CDAD appear to be well tolerated, explicit reporting of adverse events by treatment allocation is another area where future research can improve our understanding of optimal management of this disease.

Although identifying the strain of *C. difficile* is of great relevance to researchers, and can offer useful information to hospital epidemiologists, at present, strain identification is rarely performed in clinical settings. Thus, few clinicians treating CDAD are aware as to which strain of *C. difficile* is causing an individual patient's disease, and can at most make an assumption as to the strain type based on current epidemiology reported in the literature. This limitation makes any difference by strain in treatment efficacy of uncertain relevance.

Nonstandard treatment. Additional research on nonantibiotic interventions as adjunctive or alternatives to standard antibiotics for preventing and treating CDAD is needed and encouraged. Studies to prevent recurrence of *C. difficile* are a priority of prevention. As no single approach has been shown to be superior, promoting studies of different types of interventions is reasonable at this time.

Fecal biomass transplant is one novel therapy for which continued research is supported. Of all the nonantibiotic interventions, probiotics have been investigated in the most studies, and the results are not encouraging. Unlike fecal transplant, probiotics provide only a single or few strains of bacteria, and thus may be insufficient to correct alterations in the complex and extensive micorbiome to the extent needed be therapeutic. The genomic mapping of indigenous microflora may offer new information to guide future formulation of a probiotic that can effectively target alterations in the microbiome in CDAD and other diseases of the colon. A third strategy related to modifying microbial ecology in CDAD for which additional research is supported is administration of a nontoxigenic strain of *C. difficile*.

Development of a binder of *C. difficile* toxins A and B, Tolevamer, appears to build on and refine earlier approaches using less specific absorptive resins, such as choleysteramine. Tolevamer was nevertheless inferior to standard antibiotic treatment. The results suggest that additional research characterizing the action of *C. difficile* toxin in the intestinal lumen might guide the design of a more effective anti-toxin agent. A vaccine to *C. difficile* toxin to bolster the immune response to the disease is an alternative systemic approach that should be developed further.

Studies are needed to determine whether some patients might be more likely to respond to nonantibiotic interventions. Sampling in current studies of nonantibiotic interventions varies considerably, ranging from individuals who are just starting antibiotics for infections other than *C. difficile*, those who have had multiple failures of antibiotic treatment for CDAD itself, and those who have had *C. difficile* in the past. Whether any one type of nonantibiotic intervention is effective in all of these types of cases is a question. More information is needed about patients who are at high risk for recurrence of CDAD.

The effects of sequencing therapies (antibiotic as well as nonantibiotic) on the resolution of CDAD merits further research. Studies show a variety of procedures for administering probiotics to prevent CDAD, for example, such as during standard antibiotic therapy or for a period after standard treatment is completed. Determining the optimal timing to introduce nonantibiotic interventions to possibly maximize their effect is recommended.

Methodological Improvements. It is essential that future studies of a nonantibiotic intervention for treatment or prevention of CDAD be supported by a power analysis, adequate

sample size, and an intent-to-treat analysis, in addition to other standard quality components of experimental design. Multi-center studies may be necessary to achieve adequate sample sizes. Laboratory confirmation of a pathogenic *C. difficile* organism (e.g., by toxin testing) and clinical symptoms of disease (e.g., diarrhea) are essential not only for study eligibility but for determination of recurrence in long-term followup. Adoption of a standard definition of diarrhea as part of the definition of CDAD is strongly recommended. Similarly, a standard definition of CDAD resolution should be adopted. RCTs that compare more than one type of nonantibiotic intervention are suggested for efficiency.

Executive Summary Table 1. Summary of evidence

Key Questions	Level of Evidence	Summary/Conclusion/Comments
Key Question 1 Diagnostics		
Immunoassays for toxins A and B	Low to moderate	• Eight studies directly compared at least two immunoassays for toxins A and B, providing 15 pairwise comparisons of 7 difference immunoassays. Comparative data were not found for many currently used tests. • Precise estimates of the differences in sensitivity were not found nor were there any statistical differences between the tests that were compared. • Substantial differences in false positives, i.e. specificity, were not found among the tests that were compared.
Gene detection tests vs. immunoassays for toxins A and B	Low to moderate	• Three studies compared at least one gene detection test to at least one immunoassay for toxins A and B, providing a total of nine direct comparisons. Comparative data were not found for many currently available gene detection tests. • The gene detection tests can be substantially more sensitive than immunoassays for toxins A and B, with no or relatively modest loss of specificity.
2-step tests vs. immunoassays	Low	• One study compared at least one enzyme immunoassay for toxin A B to at least one two-step test using GDH as the first step and the toxin A and B immunoassay as the second step if the first was positive. • The two-step test produced fewer false positives (better specificity). However, the effect of the two-step test on sensitivity was estimated very imprecisely, but tended to be lower.
Patient characteristics	Insufficient	• Insufficient patient information for comparative data was found.
Key Question 2 Prevention		
Antibiotic use	Low	• Sixteen studies, including 6 bundled prevention practice studies, found appropriate prescribing practices are associated with decreased CDAD incidence • Harms were not reported.
Gloves	Low	• One controlled trial found use of gloves in hospital settings reduced CDAD incidence.
Disposable thermometer	Very low	• Three time series/before-after studies, two with controls, found use of disposable thermometers in hospital settings reduced CDAD incidence.
Handwashing/ alcohol gel	Very low	• No study examined whether handwashing reduced CDAD incidence. • Two studies, one controlled trial and one before/after study, of use alcohol gel to reduce MRSA transmission did not find significant differences in CDAD incidence
Disinfection	Very low	• Thirteen before-after studies of outbreaks or endemic hospital settings found intensive disinfection with a chemical compound that kills C. difficile spores reduced CDAD incidence.
Sustainability	Insufficient	• No evidence was available.
Risk factors	Very low to low	• Ten observational studies found evidence for antibiotic use, whether specific or general, increased risk of CDAD. • Severe underlying disease, acid suppression, and age are indicated as risk factors. A number of other potential factors may be indicated in single studies.
Multiple component strategies	Insufficient	• Eleven time series/before-after studies examined bundles of prevention components in a single intervention. Data is insufficient to draw conclusions. • Harms were not reported.

Key Questions	Level of Evidence	Summary/Conclusion/Comments
Key Question 3 Antibiotic Treatment		
Vancomycin vs. metronidazole	Moderate for clinical cure, low for all other outcomes	• There were three head to head trials with a total of 335 subjects. Trials used various definitions of CDAD patient and cure definitions, especially with regard to stool count and consistency. • No significant differences in outcomes, including initial cure, clinical recurrence, mean days to resolved diarrhea, were found. • Our results build upon, and are consistent with, the Cochrane Review completed by Bricker et al.[181]
Severe disease, vancomycin vs. metronidazole	Low for clinical cure, very low for all other outcomes	• One RCT examined a pre-specified subgroup of 69 subjects with severe CDAD. • Vancomycin resulted in improved clinical cure, the equivalent of 203 more cases per 1000 subjects, or roughly two cases per ten subjects.
All other comparisons of standard treatments	Very low to low	• There were seven trials examining: vancomycin vs. bacitracin (two trials), vancomycin vs. nitazoxanide, vancomycin high vs. low dose, vancomycin vs. placebo, metronidazole vs. nitazoxanide, and metronidazole vs. metronidazole plus rifampin (one each).
Strain of organism	Insufficient	• No comparative data were available.
Patient characteristics	Insufficient	• No comparative data were available.
Resistance of other pathogens	Insufficient	• No data were available.
Key Question 4 Nonantibiotic Treatment		
Treating CDAD, active control	Low	• Probiotics, prebiotics, tolevamer, C. difficile immune whey, and colestipol, are not more effective in treating CDAD than standard antibiotic treatment with oral vancomycin or metronidazole or placebo.
Treating CDAD, placebo	Low	• Administration of a probiotic with live bacteria to treat CDAD in critically-ill patients increases risk for greater morbidity and mortality from fungemia without any known benefit.
Treating Recurrent CDAD	Very Low	• There is limited very low quality evidence from two case series that fecal biomass transplant is effective in treating recurrent CDAD for up to 1 year.
Preventing CDAD	Low	• There is limited evidence that the nonantibiotic interventions in this review are not more effective than placebo for primary prevention of CDAD.
Preventing recurrent CDAD	Low to moderate	• There is limited low quality evidence from one subgroup analysis that a prebiotic may reduce diarrhea recurrence in patients treated for CDAD more so than placebo with standard antibiotics. • There is limited moderate quality evidence from one study that monoclonal antibodies are effective in preventing recurrence of CDAD

ExecutiveSummary Table 2. Future research recommendations

Key Question	Research Gaps	Types of Studies Needed to Answer Questions	Future Research Recommendation
Key Question 1. How do different methods for detection of toxigenic *C. difficile* compare in their sensitivity, specificity, and predictive values?	• Few comparisons are available. • Heterogeneity is an obstacle. • Unknown what differences in sensitivity and specificity would alter clinician decisionmaking • Unknown influence of patient and stool characteristics on test sensitivity and specificity	Comparison of diagnostic tests using same samples, same labs. Multicenter studies with well-documented patient samples.	• Document stool sample characteristics, patient selection criteria, patient characteristics, signs and symptoms of suspected CDAD.
Key Question 2. What are effective prevention strategies?	• Little evidence available with clinically important outcomes.	High quality comparative studies evaluating effectiveness and harms of single and/or multicomponent prevention strategies, including cleaning, isolation, antibiotic restriction Discrete simulation models	• Pool data from multiple participating hospital sites • Establish minimum data sets for observational data points which can inform models
Key Question 3. What are the comparative effectiveness and harms of different antibiotic treatments?	• Limited evidence available on whether vancomycin is more effective for severe CDAD.	High quality comparative studies with adequate power to detect significance in a priori subgroups	• A uniform and clinically relevant definition of severity • Subgroup analysis may include age, gender, comorbid conditions • Explicit reporting of adverse events
Key Question 4. What are the effectiveness and harms of nonantibiotic adjunctive interventions?	• Probiotics as a treatment adjuvant is not supported. Potential harms to seriously ill patients may outweigh potential benefits for further prevention research. • Probiotics as prevention warrants further study. • Further research of monoclonal antibodies for prevention is warranted • Further research of fecal transplant is warranted	High quality comparative studies with adequate power.	• Placebo comparators would contribute indirect evidence that would help guide potential combination therapies. • Quality research includes power analysis, intention to treat • Multicenter trials are likely needed to achieve adequate samples • Trials of probiotics for prevention are well represented in ongoing studies • Patient characteristics for sub-group analysis
Umbrella issues			• Adoption of standard definitions for diarrhea, CDAD resolution

Evidence Report

Chapter 1. Introduction

Background

C. difficile-associated disease (CDAD) is an important hospital-acquired infection and a growing health care problem. *C. difficile* is a gram-positive, spore-forming, anaerobic bacterium that, when ingested, can cause CDAD if it is a toxigenic strain. CDAD symptoms can range from mild diarrhea to severe cases including pseudo membranous colitis and toxic megacolon. CDAD incidence is estimated at 6.5 cases per 10,000 patient days in hospital.[1] About 250,000 hospitalizations were associated with CDAD in 2005.[2] Mortality from CDAD is estimated at 7 percent of cases.[3] The vast majority of severe morbidity and mortality is experienced by elderly people in hospitals.[4-6] Residents of long-term care facilities (LTC) are also at higher risk, with up to 26.2 cases per 10,000 resident days versus 6.5 cases per 10,000 hospital days.[7] Incidence rates may increase by four or five fold during outbreaks.[8] In addition to institutional care environments, *C. difficile* is also common in the community, being easily isolated from soil and water samples.[9] Community associated CDAD rates are generally much lower, accounting for 27 percent of all CDAD cases in a recent prevalence study[8] but are also on the rise.[10] However, the source of the *C. difficile* organisms responsible for cases of CDAD in the community is not well understood.

In order for CDAD to develop, a person must be infected with a strain of *C. difficile* capable of making toxin in the person's colon (see Figure 1). Toxigenic strains include those that make toxin A (a cytotoxin), toxin B (an enterotoxin), or both toxins. Infection with both toxigenic and nontoxigenic strains commonly occurs in healthy individuals. If these persons have usual, healthy colonic flora, the risk of CDAD is exceedingly low. There is a small risk of CDAD if the colon flora becomes disturbed, commonly through antibiotic use, while the person is infected (colonized) with a toxigenic strain. Antibiotics that disturb colon flora enough to allow CDAD to develop must get into the colon, and they are associated with profound alterations in relative amounts of colon bacterial constituents.[11,12] The immune status of the patient also contributes to the risk of developing CDAD and the experienced severity.[13] Other risk factors include increasing age, female gender, comorbidities, gastrointestinal procedures, and use of gastric acid suppressant medications (although the last is still controversial).[14-18] Risk profiles for recurrent CDAD are similar.[15] One study, which statistically modeled CDAD within the hospital setting, suggested that reducing patient susceptibility to infection is more effective in reducing CDAD cases than lowering transmission rates.[19]

New, more virulent strains have emerged since 2000. Characteristics associated with hypervirulent strains include increased toxin production (due to a deletion in a toxin regulatory gene), an additional binary toxin, whose role in disease etiology is not well understood, hypersporulation, and high-level resistance to fluoroquinolone antibiotics.[20] These new strains affect a wider population, often people with a lack of established risk factors for CDAD based on older strains, such as previous hospitalization or antibiotic use, and include children, pregnant women, and other healthy adults.[21] With hypervirulent strains the time from symptom development to septic shock may be reduced, making quick diagnosis and proactive treatment regimens critical for positive outcomes.

The highly virulent strain associated with the epidemic of CDAD described in the early 2000s may be decreasing in prevalence in limited locations.[22] Recent analysis of an archived

collection of *C. difficile* isolates revealed that predominant strains shifted from year to year among a population served at a single institution,[23] suggesting that this strain shift may occur on a larger scale. However, this phenomenon potentially cuts both ways as strains drift toward lesser or higher virulence, and the possible future risks and costs of CDAD remain significant.

Diagnosis

Effective prevention of transmission and treatment of CDAD depends on swift and accurate diagnosis. None of the risk factors or clinical signs and symptoms alone or in combination, except possibly a documented presence of pseudo membranous colitis, is sufficient to surmise with a high degree of clinical certainty that a patient does or does not have CDAD. Stool culture and cultured cell cytotoxicity assay are historically held as the standard reference tests; however, results can take up to 48 hours, and these diagnostic methods require a level of expertise and equipment that are not widely available. A number of faster, less demanding diagnostic tests have been developed to detect either the presence of toxins produced by most *C. difficile* organisms, toxins A and/or B, or the genes involved in the production of, or regulation of, toxins A and/or B. These tests have a variety of sensitivities, specificities, biotechnologies used, costs, and time-to-results. The sensitivities and specificities of the newer tests have been studied mostly using toxigenic culture or a cultured cell cytotoxicity assay as the reference test, but the estimates vary substantially, making it difficult to determine whether there are clinically significant differences between tests.[88,89] Greater than 90 percent of labs in the United States use one of the commercially available immunoassays to detect toxins in stool samples or colonies of cultured organisms, because they are fast, inexpensive, and technically easier to perform.[95] A more detailed discussion of types of diagnostic tests for *C. difficile* is provided in a supplemental section at the end of this chapter.

When evaluating laboratory tests for the presence of toxigenic *C. difficile* in patients, it is important to consider how patients were selected, and the consistency of the stool specimens being tested. Testing for *C. difficile* infection is recommended for a person with diarrhea (generally three or more loose or unformed stools for 1 to 2 days) and one or more risk factors for CDAD.[96,97] However, these recommendations may not always be followed in practice. Several multivariable prediction models built on established risk factors have been published in an effort to optimize diagnostic testing for *C. difficile* infection.[98-100] The extent of their use in clinical practice is not known.

Identifying the most accurate diagnostics tests in clinical practice could be very important to clinical practice. Diagnostic tests with greater sensitivity (fewer false negatives) would reduce the number of patients who do not receive appropriate treatment and isolation. Tests with higher specificity (fewer false positives) could reduce the number of unnecessary and potentially detrimental interventions, such as withholding antibiotics for other medical conditions, or initiating treatment for CDAD. Swift diagnosis leading to infection prevention precautions, faster treatment, and quicker resolution of diarrhea may reduce the amount of organisms or spores in the environment that can infect other patients.

Treatment

There are a number of algorithms available to guide treatment of CDAD.[10,101-103] The only antimicrobial currently approved for the treatment of CDAD by the United States Food and Drug

Administration is oral vancomycin, and consensus appears to exist for treatment of severe initial incident CDAD with vancomycin. However, there also appears to be clinical consensus to treat mild to moderate CDAD with metronidazole, in part because of the concern that overuse of vancomycin may contribute to increasing pathogen resistance[104] and cost considerations. Pepin[103] suggests that both vancomycin and metronidazole are implicated in increased frequency of vancomycin-resistant enterococci (VRE). Enterococci are part of the normal GI flora, and VRE are a major problem. Whether the increased use of vancomycin for CDAD will affect the rates of VRE is unclear, especially as increased density of VRE in stool has been demonstrated in subjects receiving antimicrobials active against anaerobes (the main colonic flora), including both oral vancomycin and metronidazole.[105] Surgical treatment with colectomy can be life saving in patients with fulminant, or acute severe, colitis.[106]

Nonantibiotic interventions for the treatment and prevention of CDAD have been sought for several reasons. Treatment with standard antibiotics, such as vancomycin and metronidazole, is ineffective in 25 to 30 percent of patients with CDAD,[64] no antibiotic kills *C. difficile* spores, and rates of infection are increasing. Treatment for relapsed or recurrent CDAD is much more problematic. CDAD recurs in about 20 percent of patients;[101] a subset of recurrent patients spiral into several subsequent recurrences.[107] Clinicians have chosen from a number of antibiotics and dosing protocols, and adjunctive treatments, such as the use of antimicrobials, probiotics, fecal transplant, toxin-binding agents, and immune-system enhancing agents.[108-110] The emergence of the new, more resistant strains of *C. difficile* encourages nonantibiotic approaches to avoid potential future "super resistance."

Probiotics are a very active area of discussion for CDAD.[111] Probiotics are living microorganisms, including bacteria or yeast, which is believed to restore microbial balance to gastrointestinal flora when administered in adequate amounts. It is important that the effectiveness of probiotics and related substances are evaluated specifically for their effect on CDAD and not rely on the more broadly defined antibiotic-associated disease (AAD), which includes a much broader set of potential disease etiology.

Prevention

Prevention of CDAD takes two general forms, breaking routes of transmission and improving a patient's resistance to disease should infection occur. Preventing the spread of *C. difficile* by breaking routes of transmission within institutional settings is dependent on staff compliance with national guidelines and standards[112] and locally determined hygiene protocols. *C. difficile* is common in the environment of people with CDAD,[91] most of whom have diarrhea, and many of whom have incontinence and often other medical problems that tend to diminish personal hygiene. *C. difficile* is found on the hands of hospital workers[39,91] and is more likely to be found on hands of people who have been working in a heavily contaminated room.[91] Thus, *C. difficile* acquired in hospital settings may be spread directly or indirectly from patient to patient.[93]

Complicated recommendations are difficult to remember and implement, and protocols for different targeted hospital acquired infections are not always congruent. For example, the availability of alcohol hand rubs improved physician compliance and reduced Methicillin resistant Staphylococcus aureus (MRSA) infections,[44] yet *C. difficile* produces spores that can withstand hostile environments and are resistant to alcohol hand rubs and other routine antiseptics. One concern has been that health care workers will use alcohol based rubs or gels in

circumstances where hand washing is preferred. Other institutional prevention strategies may be required as *C. difficile* transmission knowledge develops. For example, a recent study isolated *C. difficile* spores from air samples in a hospital in the United Kingdom 4 to 7 weeks after the last confirmed CDAD case in the ward, and successfully cultured bacterium from the spores.[113]

Scope of the Review

The purpose of this systematic review was to provide an overarching assessment of the evidence for comparing the accuracy of diagnostic tests, and the effectiveness of prevention and treatment interventions on initial and recurrent CDAD related patient outcomes in adult patients. This purpose was developed during the project's topic refinement stage. There was consensus among key informants that the single greatest contribution this systematic review could contribute to the field was to provide a comprehensive review by an independent organization that covered the major concerns of the field. CDAD is an active topic in the literature as well as a vital clinical concern. The consensus opinion included the idea that clinicians and researchers both would be well served to reaffirm what is and is not supported by evidence in the literature and at what level of evidence, to balance against this activity level.

The general use of the term *C. difficile* associated disease, or CDAD, rather than *C. difficile* infection (CDI) underlines the major impetus of this review, which is concern for the presence of clinical disease, not asymptomatic carriage of the *C. difficile* organism. While we were interested in how treatment of CDAD varies by organism strain, molecular epidemiology studies whose main purpose was to identify the strains of *C. difficile* present in the population are also outside the scope of this review. The review focuses on adult patients because adults, and particularly elderly adults, carry the large majority of the morbidity and mortality burden.

Key Questions

The following key questions form the basis for this review:

Key Question 1. How do different methods for detection of toxigenic *C. difficile* to assist with diagnosis of CDAD compare in their sensitivity and specificity?
(a) Do the differences in performance measures vary with sample characteristics?

Key Question 2. What are effective prevention strategies?
(a) What is the effectiveness of current prevention strategies?
(b) What are the harms associated with prevention strategies?
(c) How sustainable are prevention practices in health care (outpatient, hospital inpatient, extended care) and community settings?

Key Question 3. What are the comparative effectiveness and harms of different antibiotic treatments?
(a) Does effectiveness vary by disease severity or strain?
(b) Does effectiveness vary by patient characteristics: age, gender, comorbidity, hospital versus community acquired setting?
(c) How do prevention and treatment of CDAD affect resistance of other pathogens?

Key Question 4. What are the effectiveness and harms of nonantibiotic adjunctive interventions?
(a) In patients with relapse/recurrent CDAD?

Review Framework

The conceptual framework which guided this review is provided in Figure 2. The figure lays out the clinical path for patients with the potential to develop CDAD, from diagnostic laboratory tests, through their impact on treatment decisions, to finally implications for prevention strategies and locates the key questions of this review within the context of the framework. Diagnostic testing has two parts, the technical efficacy of the tests and diagnostic accuracy. Technical efficacy is outside the scope of this review; rather, for Key Question 1 we focus on the comparative diagnostic accuracy of commonly used rapid tests, such as immunoassays for *C. difficile* toxin and toxin gene detection tests, which may reduce the time lapse between patient symptom onset and laboratory confirmation of CDAD and treatment decisions. Repeat testing of selected specimens does not provide good comparative information about test accuracy and therefore is not covered in the focused review of diagnostic test accuracy. When a patient is treated for CDAD, whether for an initial case, a relapse, or recurrence, the clinical outcomes of interest establish the patient treatment efficacy. Of particular interest, Key Question 3 will compare effectiveness of established treatments used for CDAD, particularly vancomycin and metronidazole. For Key Question 4, the clinical question of interest is what nonstandard treatments are being utilized, and their efficacy, particularly for recurrent CDAD. After diagnostic accuracy, and treatment, and patient outcome efficacy concerns, prevention, is a societal level efficacy measure, as the benefits of prevention of infectious disease can extend beyond the individual patient. This is the area of focus for Key Question 2. Key Question 4 also contributes to this area to the extent that nonantibiotic treatments assist a patient in fending off an infection.

Figure 3 expands the framework for the key question related to prevention. The illustration lays the pathway of preventive strategies and practices from the target patient population of patients at risk for CDAD due to potential for exposure, through intermediate outcomes and on to health outcomes. This framework was included to highlight the linkage but conceptual difference between the intermediate outcomes of prevention, which are often process measures of the uptake of a prevention strategy, or counts of vegetative *C. difficile* or spores remaining in the environment, and health outcomes of clinical significance important to the patient. Key Question 2 is mainly concerned with evidence for the direct effect of prevention on health outcomes.

Figure 1. Pathogenesis of CDAD

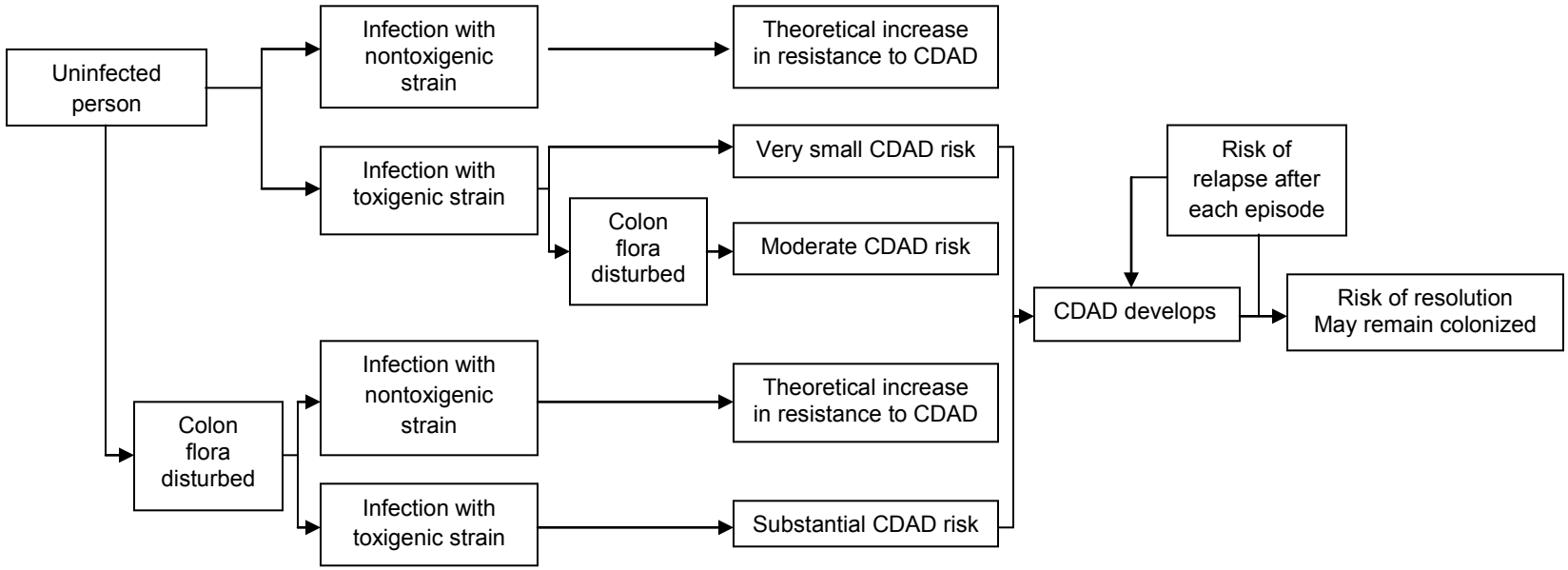


Figure 2. Analytic framework for CDAD diagnostic testing, prevention, and treatment

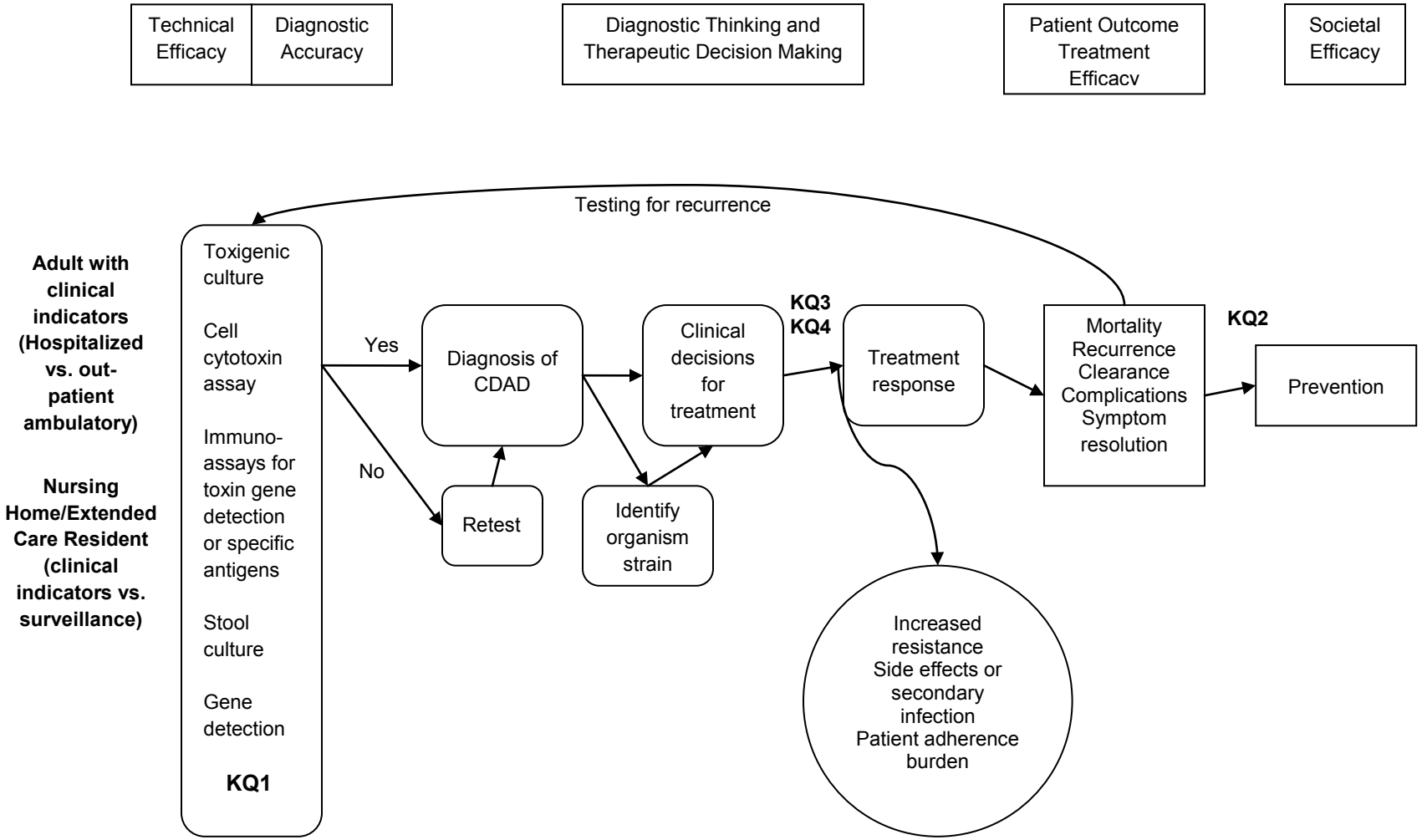
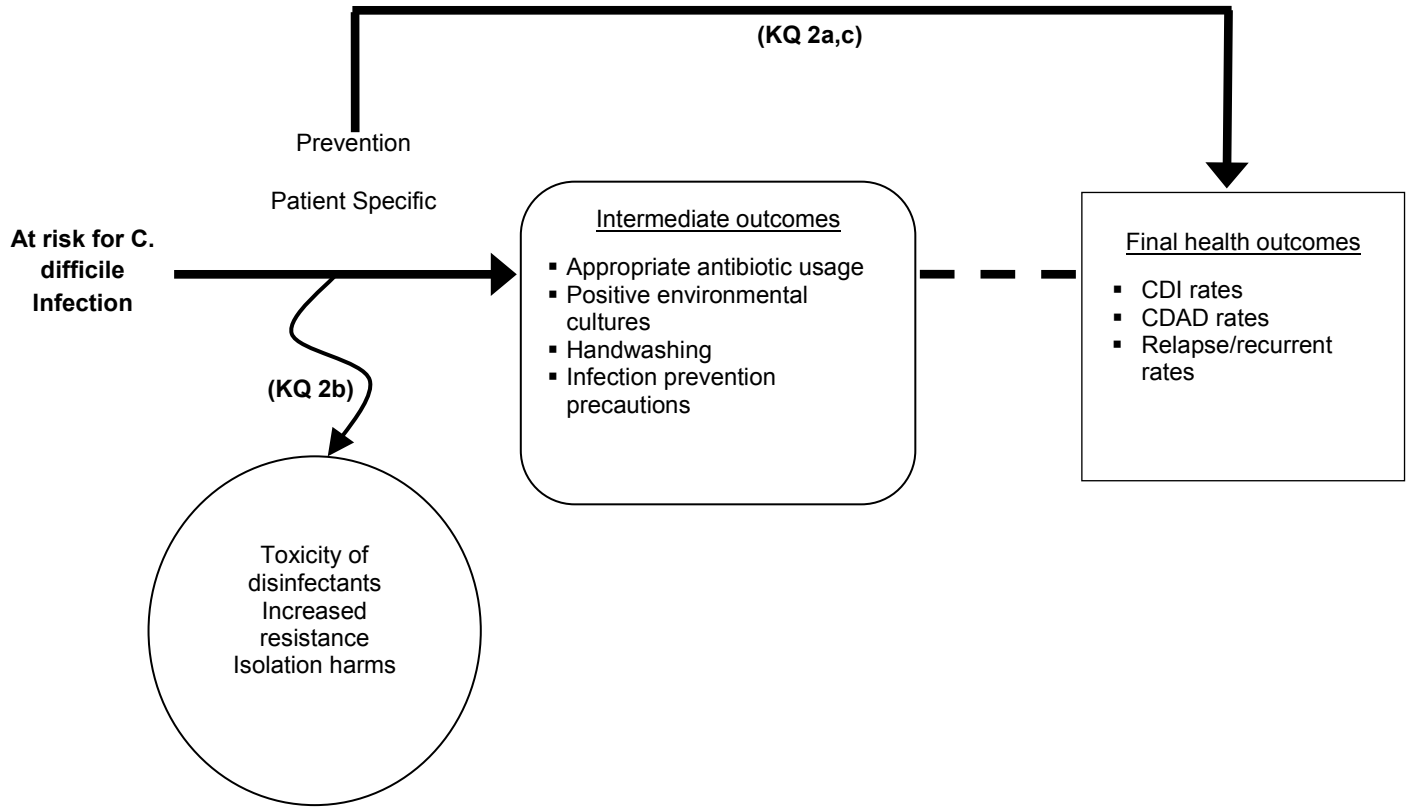


Figure 3. Supplemental prevention framework



Glossary of Terms

Carrier – asymptomatic person colonized by C. difficile

CDI - *C. difficile* infection. This term applies to the presence of C. difficile in a patient confirmed by laboratory tests. The term itself does not necessarily imply the patient has a clinical disease or that the infection produces the toxins believed to cause the disease. If tests indicate that the CDI can produce the toxins that cause the diarrhea and other signs and symptoms, it is termed toxigenic CDI.

CDAD – *C. difficile*-associated disease. This acronym is also commonly used in the literature for *C. difficile*-associated diarrhea. We chose the more expansive term "disease" as CDAD symptoms are broader in nature.

Colonization – – *C. difficile* becomes established in intestine. Individual may or may not have CDAD.

False Negative Fraction - fraction of tested stool specimens that had a positive reference test and a negative result for the diagnostic method being evaluated. The false negative fraction is equal to one minus the sensitivity.

False Positive Fraction - fraction of tested stool specimens that had a negative reference test and a positive result for the diagnostic method being evaluated. The false positive fraction is equal to one minus the specificity.

Gene detection test – methods of amplying (replicating) specific parts of genetic material (DNA) in samples usually using the polymerase chain reaction (PCR) followed by detection of the highly replicated gene fragment.

Hypervirulent – strains of CDAD that include increased toxin production, an additional binary toxin, hypersporulation, and high-level resistance to fluoroquinolone antibiotics.[20]

Hypersporulation– accelerated rate of spore production.

Immunoassay – test that is based on interactions between added animal antibodies against the specific substance to be detected, e.g. *C. difficile* toxins or glutamate dehydrogenase. Different tests use varying methods to isolate and detect the antibody/antigen complexes formed in the specimen being tested. Commonly referred to as enzyme immunoassay (EIA) in the literature.

Negative Predictive Value – fraction of tested stool specimens that were negative for the diagnostic method being evaluated and negitve on the reference test. Depends on the prevalence of the disease in the tested specimens.

Non-standard therapy – therapies other than treatment with antibiotics for CDAD, such as probiotics, prebiotics, monoclonal antibodies, and fecal transplants.

Positive Predictive Value – fraction of tested stool specimens that were positive for the diagnostic method being evaluated and positive on the reference test. Depends on the prevalence of the disease in the tested specimens.

Prebiotics – non-digestible foods that create environments healthy for bacteria growth.

Probiotics – - living microorganisms, including bacteria or yeast, which are believed to restore microbial balance to gastrointestinal flora when administered in adequate amounts

Recurrent CDAD – recurrence of symptoms within 8 to 10 weeks after sessation of specific antibiotic therapy, with exclusion of other enteropathogens and a positive diagnostic test for toxigenic C. difficile.[107]

Sensitivity – fraction of tested stool specimens that had a positive reference test and a positive result for the diagnostic method being evaluated. Also known as the true positive fraction.

Severe CDAD – Definitions vary in the literature, but generally refer to a CDAD diagnosis in combination with more complex manifestations of disease or in patients with significant other risk-factors (age, signs of infections, comobidities).

Specificity – fraction of tested stool specimens that had a negative reference test and a negative result for the diagnostic method being evaluated. Also known as the true negative fraction.

Diagnostic Test Descriptions

Cytotoxicity assay. The cultured cell cytotoxicity assay is often used as a reference test for evaluating new diagnostic tests for *C. difficile* toxin. Briefly, a diluted and filtered aliquot of a stool sample is mixed with cultured test cells. The test cells are examined for toxin effects (cell rounding) that are not seen in comparator test cells where an excess amount of antitoxin is present.[114] The diagnostic rounding of cultured test cells and the clinical signs and symptoms of CDI can be caused by cellular interactions with both *C. difficile* toxins although toxin B is much more cytotoxic and the cytotoxicity assay is often considered to be a test for toxin B.[115,116] A cytotoxicity assay requires up to 48 hours for the toxin effects to appear especially when toxin level in the test material is low. Cytotoxicity testing is not standardized or a perfectly accurate reference method.[117] Methodological differences in the storage, time to process and dilution of stool samples, the age and type of cultured test cells being used for the test, the antitoxins, and in the interpretation of results all can cause cytotoxicity assay results to vary.[118] Toxins can degrade or be inactivated depending on how long fecal specimens are stored before being tested and the storage temperature. Nevertheless, the imperfect cytotoxicity assay is often used as the standard reference method in the evaluation of other diagnostic tests for CDI.

Detection of *C. difficile* organisms. Culturing *C. difficile* by anaerobic incubation of fecal aliquots on selective cycloserine-cefoxitin, fructose agar or other media can be more sensitive than the cytotoxicity assay for detecting the presence of *C. difficile* organisms.[118,119] However, *C. difficile* culture techniques also are not standardized, are susceptible to methodological variation, and require expertise, equipment, and several days to complete. Furthermore, cultured *C. difficile* organisms need to be tested to determine whether they can produce disease-causing toxins

because many individuals may be carriers of *C. difficile* organisms that do not produce toxins or clinically significant CDI. Nevertheless, expert culture of *C. difficile* from stool samples followed by a cytotoxicity assay or another method of detecting toxins is considered the most sensitive method for detection of toxigenic *C. difficile*, albeit not very practical.[97,120] However, the concentration of toxins produced in culture, might not be the same as present in patients.

Assays for glutamate dehydrogenase enzyme constitutively produced by *C. difficile* have been used as a faster and less demanding alternative to culturing *C. difficile* organisms. These tests are not entirely specific because other organisms can produce glutamate dehydrogenase or interfering substances.[94,118] Like stool cultures, a positive glutamate dehydrogenase test requires a second test to detect *C. difficile* toxins. Because fecal cultures and the cytotoxicity assay are demanding, costly, and time consuming, and most stool samples sent to clinical laboratories turn out to be negative for toxigenic *C. difficile*, some laboratories have proposed using a test for glutamate dehydrogenase first, and then testing only the positive specimens for toxins.[121-123] In this two stage approach, a negative test for glutamate dehydrogenase would preclude the need for a toxin test. However, the sensitivities of glutamate dehydrogenase assays need to be high enough to have an acceptably low number of false negatives.[96] Furthermore, the performance of a two stage test also will depend on the sensitivity and specificity of the second test used to detect toxin.

Immunoassays for toxins. A variety of faster (within a few hours), less costly commercial immunoassays for *C. difficile* toxins have been developed and have been commercially available since the late 198's. Initially most immunoassays detected only toxin A. More recently it was discovered that a small but increasing number of clinically significant *C. difficile* strains produced only toxin B.[124-127] The incidence of clinically significant toxin A-negative, B-positive organisms in the United States is not known and could vary by site and time.[128] When the performance of a diagnostic test depends on the level of toxins in test specimens and most organisms produce both toxins A and B, immunoassays that detect both toxins might be more sensitive if other critical factors such as dilution of the specimens are equal.[129] Therefore, experts have recommended using immunoassays that can detect both toxins A and B.[96,97,117,128] A highly sensitive and specific immunoassay for toxin might be used as a second test after either stool culture or the glutamate dehydrogenase assay rather than a cytotoxicity assay.

Data from the College of American Pathology proficiency testing program for *C. difficile* toxin detection indicated that 90 percent of labs used an immunoassay for toxins A and B in June 2009. The most commonly used tests were the Meridian Immunocard and Premier A & B, the TechLab Tox AB II and Toxin A/B QUIK CHEK kits, and the Remel ProSpecT and Xpect Toxin A/B tests. These data are consistent with an online survey of members of the Association for Professional Infection Control and Epidemiology in 2008 that indicated that an immunoassay was used in 95 percent of patients who were diagnosed with CDAD in 648 responding American laboratories, and 60 percent were diagnosed using an immunoassay for toxins A and B, while only 3 percent used an immunoassay for only toxin A.[8]

Toxin gene detection tests. Several tests of stool specimens for the presence of genes involved in the production of *C. difficile* toxins have recently become commercially available. These tests use the polymerase chain reaction to amplify (replicate) targeted gene fragments to detect the presence of a gene or genes involved in the production of toxins, not the actual toxins. The target of the assays can be the genes that produce toxin A and/or B or a gene C that negatively regulates the production of toxins A and B. A mutation in gene C has been detected in an increasingly common hypervirulent strain of *C. difficile* that produces large amounts of toxins

A and B.[128] One concern about using the tests based on amplification of toxin gene fragments is that very small, clinically unimportant genetic residue or specimen contamination may be detected. Clinically speaking these would be false positives. Therefore, it might be important to use this type of test only when a patient has clinical symptoms suggestive of CDI.[96,118]

Chapter 2. Methods

Topic Refinement

The topic for this report was nominated in a public process. We drafted the initial key questions with input from a key informant panel composed of researchers, clinicians, professional organizations representing hospitals, infectious diseases, and clinicians, federal and state agencies, patient safety advocates, and consumers. After approval from AHRQ, the key questions were posted to a public Web site. The public was invited to comment on these questions. After reviewing the public commentary and conferencing with the Technical Expert Panel (TEP) (Appendix A), we drafted final key questions and submitted them to AHRQ for approval.

Systematic Review

Search Strategy

Our search strategy used the key word "*difficile*" to identify all articles related to *C. difficile* because we found the keyword to be a more sensitive term than the National Library of Medicine's Medical Subject Headings (MeSH) keyword nomenclature. Articles were limited to English language, humans, and MeSH filters for adult populations. We searched MEDLINE®, AMED, the Cochrane library, and ClinicalTrials.gov. Details of the major search strategies are provided in Appendix B.

To identify systematic reviews, we searched MEDLINE®, the Cochrane Database of Systematic Reviews, and the Web sites of the National Institute for Clinical Excellence, Guidelines.gov, and the NHA Health Technology Assessment Programme. We used results from previously conducted meta-analyses and systematic reviews when appropriate. We also manually searched reference lists of review articles and articles that were read for the review. All citations were imported into Refworks for initial screening, and then EndNote X for database management.

During the manual search of included articles' reference lists, we found a number of studies not identified in our original search. We performed a forensic examination of those missed articles and determined that diagnostic test and prevention articles in particular were often not indexed by patient ages. We therefore performed a second search without the age filters. These search strategies are also included in Appendix B.

We conducted the initial searches in October 2009. The no-age filtered searches were conducted in February 2010 and updated in March 2010.

Inclusion/Exclusion Criteria

In brief, we developed criteria for inclusion and exclusion of studies based on the patient populations, interventions, outcome measures, and types of evidence specified in the key questions. We retrieved full-text articles of potentially relevant abstracts and conducted a second review for inclusion by reapplying the inclusion criteria. Results published only in abstract form

are generally not included in our reviews unless adequate information was available to assess the validity of the data. Full details by key question are provided below.

Key Question 1.

Patients. We restricted the review to studies that used clinical stool specimens from patients suspected to have CDAD. Information that described patient characteristics that could be related to CDAD, hence test performance, was of particular interest.

Study selection. We sought studies that concurrently compared at least two diagnostic tests in the same laboratory using the same stool samples from cases of clinically suspected CDI using the same reference standard. This was done in order to reduce the heterogeneity in the estimates of differences in sensitivity and specificity, given the inter- and intra-laboratory variation in the application of diagnostic tests for CDI, reference standards, and differences in patient and stool specimen characteristics. Diagnostic tests of interest were the immunoassays commonly used in the United States to test for the presence of both toxins A and B, newer tests to detect the presence of *C. difficile* gene fragments involved in the production of toxin and 2-step testing methods that first use an immunoassay for glutamate dehydrogenase enzyme (GDH), to detect *C. difficile* organisms, in conjunction with a second test for the presence of toxin. We did not include articles that only compared tests that are not currently commercially available in the United States. We focused on tests for toxigenic CDI because the presence of toxins is a requisite for diagnosing clinical disease or CDAD.

Measures of diagnostic accuracy. We sought to compare diagnostic tests in terms of differences in their sensitivity (true positives for toxigenic CDI) and specificity (true negatives for toxigenic CDI). These statistics are believed to be most relevant to clinical decisionmakers. To be consistent with other common statistical analyses, such receiver operator characteristic curves and likelihood ratios, we present and discuss study results in positive terms, that is, true positives (sensitivity) and false positives (1-specificity). The review was restricted to studies that used toxigenic culture, cell cytotoxicity assay, or combinations of tests, as the reference test for the presence or absence of toxigenic CDI. To be able to compare estimates of sensitivity and specificity, the report had to provide the counts of test results for those that were positive or negative according to the reference test. Direct comparisons of diagnostic tests without a reference test were not included.

Key Question 2.

Patients. We included studies targeting adult patients at risk for exposure to *C. difficile* in hospital and long-term care facilities.

Interventions. We included studies that examined the effects of prevention strategies aimed at (1) breaking routes of transmission within institutional settings, the major focus of institutional infectious disease programs, and (2) reducing susceptibility to CDAD through antibiotic prescribing practices. Reducing susceptibility to CDAD through other agents is covered in Key Question 4.

Comparators. No restrictions were placed on the comparators, although we anticipated that most studies would use some form of usual processes of care.

Outcomes. We included only studies with CDAD incidence, or other measures of CDAD as an outcome. We excluded studies that used only process measures, or intermediate outcomes, such as reduced spore count in environmental samples, and did not tie these measures to CDAD incidence. We looked for harms including difficulties experienced by employees responsible for environmental cleaning, or overtreatment harms, such as increased exposure risk to CDAD if a patient without CDAD is located in an isolation ward. We also sought evidence for how well

prevention strategies and practices can be sustained past a study period, or a period of intensive effort and monitoring

Study designs. Accepted study designs included randomized controlled trials (RCT), prospective cohort, retrospective cohort, time series, and before/after trials.

In addition to studies examining prevention practices, we also identified good quality studies that identified specific risk factors for development of CDAD to facilitate infectious disease control efforts to target likely effective preventive strategies. Inclusion criteria were: (1) prospective study design; (2) the methods for the risk factor analysis were specified; (3) the study included a clearly defined control group; (4) the study was of risk for CDAD, not *C. difficile* infection or colonization; and (5) the CDAD definition included diarrhea and a positive test for *C. difficile* toxin. We included studies in which the influence of confounding variables was minimized in one of three ways: (1) randomization; (2) possible confounding variables were controlled in case and control selection process, or (3) multivariable analysis was done to determine the relative contribution of each potential risk factor included in the study.

Key Question 3.

Patients. We included target populations of adult patients with clinical signs consistent with CDAD in hospital, outpatient, or long-term care settings. We also looked for studies assessing efficacy when stratified by disease severity or strain, or by patient characteristics such as age, gender, comorbidity, and location of disease acquisition.

We sought studies which examined differences in treatment effect by disease severity. We did not exclude any studies based on the definitions they used for disease severity. In mild disease, discontinuation of the inciting antibiotic may be sufficient to resolve the symptoms of CDAD,[102,130] making it difficult to detect any difference in the efficacy of antimicrobial therapy. In severe disease, differences in treatment efficacy are easier to detect, and are of more importance because of the high morbidity and mortality associated with severe CDAD.[131] However, a major difficulty with stratifying therapy by disease severity is the lack of a standardized, reproducible, and validated tool for measuring severity.[97,132] Elements that have been incorporated into various severity definitions include age, degree of leukocytosis, fever, ileus, endoscopic findings, presence of fecal leukocytes, and need for intensive care unit (ICU) treatment or colectomy.[62]

We also sought studies that examined the comparative effectiveness of the antimicrobial treatments by organism strain. We also sought evidence of the potential impact of CDAD treatment on developing antibiotic resistance in other infectious pathogens. There has historically been reluctance to use vancomycin as a first-line drug for CDAD because of the drug's important role in treating serious bacterial infections, especially drug-resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. However, with the increase in CDAD incidence and severity,[3,133] and a randomized trial reporting superiority to metronidazole in treating severe CDAD,[62] this reluctance has been largely overcome. This may also be due to the fact that *S. aureus* and *S. pneumoniae* are not part of the usual gastrointestinal (GI) flora, so there is a plausible biological mechanism to suggest that vancomycin therapy for CDAD may not select for resistance in these clinically important pathogens.

Interventions. We sought studies which tested vancomycin, metronidazole, bacitracin, nitazoxanide, rifaximin, and rifampin, which has only been studied as an adjunct to other active drugs. As fusidic acid and teicoplain are not currently approved for use in the United States, these treatments were excluded.

Comparators. We sought studies that compared two active antimicrobial treatments, although we accepted studies that included placebo as the comparator for the two antimicrobials of interest, vancomycin and metronidazole.

Outcomes. We included initial cure, recurrence (variably defined by symptoms with or without a positive test for *C. difficile*), and mortality, which are outcomes of interest to clinicians and are reported in most studies. We also included time to resolution of diarrhea, which may be important because of effects on patient comfort, duration of hospitalization, and for infection control purposes. While we included clearance of the organism or toxin where reported, it is an outcome of uncertain significance if it is used without taking into account the patient's clinical status. We included any reported harms to patients using any of the standard antimicrobial treatments.

Study designs. We also included RCTs, prospective cohort or case control studies, retrospective cohort studies, and case control study designs.

Key Question 4.

Patients. We included target populations of adult patients with clinical signs consistent with CDAD in hospital, outpatient, or long-term care settings. Patients with relapsing or recurrent CDAD are of special concern due to the demonstrated difficulty with permanent cure of the infectious organism, and are often the stated targeted patient population for nonstandard treatments. Likewise, preventing recurrence is an important clinical goal. We sought studies which examined either preventing or treating relapsing or recurrent CDAD, as the target population or a specified subgroup. We accepted both a priori and post hoc subgroup analysis.

Interventions. We included all studies which examined any nonantibiotic treatment. Nonantibiotic treatments include a broad range of treatments, such as antimicrobial agents, agents that bind the toxins produced by *C. difficile*, or treatments which reduce a patient's susceptibility, from prebiotics or probiotics that support the gut flora to vaccinations or antibodies to enhance immune functions. We did not limit studies to a particular set of nonantibiotic treatments and instead sought to catalogue the range of treatments.

Comparators. We included studies that used either another active treatment, such as metronidazole, or placebo.

Outcomes. We examined patient outcomes, such as resolution of symptoms, for treatment studies and CDAD incidence and presence of toxins for prevention studies. We sought evidence for harms associated with nonantibiotic interventions, whether for treatment or prevention, such as side effects or secondary infections

Study designs. We anticipated few controlled trials for newer treatments and so included all study designs. We did not limit comparators for nonstandard treatments; however, we did exclude studies on nonhuman, in vivo, and healthy volunteers.

Study Selection

Results of the literature search were imported to a bibliographic database for screening. At least two independent reviewers examined all titles and abstracts for eligibility based on the inclusion/exclusion criteria. Titles and abstracts with insufficient information to determine eligibility were pulled for full article text review. Disagreements between reviewers were resolved through consensus. Final results of the screening process were then imported to an EndNote file for database management.

Data Extraction

We extracted the following data from included trials directly into study tables: study design; setting; population characteristics (including sex, age, ethnicity, diagnosis); eligibility and exclusion criteria; characteristics of the interventions; numbers screened, eligible, enrolled, and lost to followup according to the research design; method of outcome ascertainment; study quality items; and results for each outcome. All tables were subject to a quality check of all data items by independent reviewers.

Quality Assessment

Key Question 1. To assess the quality of reports for diagnostic studies, we used the criteria developed for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS).[134,135] These criteria for include: (1) tested specimens (patients, stool) and their selection were clearly described and representative of those that are tested in clinical practice, (2) the time period and handling of specimens between tests most likely did not change what is being measured, (3) all test procedures were adequately described and replicable, (4) the same credible reference test was used for all specimens, performed regardless of other test results, (5) the reference and diagnostic tests being evaluated were conducted and interpreted independently of each other, (6) any clinical information that was used in the interpretation of test results was reported, (7) indeterminate results were reported and analyzed in a reasonable manner, and (8) excluded test results, specimens or patients were reported and explained. This quality assessment does not have a method for scoring the criteria or reliably categorizing the studies. Some studies that did not meet a key criterion for inclusion in the review were excluded without further assessment of their quality.

Studies that are summarized in this review were rated as having 'good' internal validity. Comparisons were made in the same laboratory using the same specimens and a credible reference standard. There were no major differences in the processing and storing of the specimens between tests that were independently conducted. Indeterminate results were discussed and handled in a reasonable manner.

Key Question 2. Quality assessment for nonrandomized studies used primarily in assessing prevention strategies was based on study design (case control versus case series), the selection of cases or cohorts and controls (how well matched), and adjustment for confounders. Studies were rated as higher quality if they met the following a priori defined criteria: (1) prospective, (2) had explicitly detailed the methods of their study, (3) patients were representative of typical CDAD patients, and (4) used multivariate analysis to isolate the effect of the variable in question.

Key Questions 3 and 4. We rated the study quality of individual randomized controlled or clinically controlled trials using criteria based on Cochrane Collaboration recommended domains.[136] These domains assess the risk of bias of studies included in a systematic review. The first domain is adequate allocation concealment, based on the approach by Schulz and Grimes.[137] The second domain regards blinding methods, e.g., participant, investigator, or outcome assessor. The third domain regards how incomplete data are addressed; did the study analyze the data based on the intention-to-treat principle (i.e., were all subjects who were randomized included in the outcomes analyses) and were reasons for dropouts/attrition reported.

Studies were rated to be of good, fair, or poor quality. A rating of good generally indicates that the trial reported adequate allocation concealment, blinding, analysis by intent to treat, and

reasons for dropouts or attrition. Studies were generally rated poor if the method of allocation concealment was inadequate or not defined, blinding was not defined, analysis by intent to treat was not utilized, and reasons for dropouts or attrition were not reported and/or there was a high rate of attrition.

Rating the Body of Evidence

For randomized trials, the overall strength of evidence was evaluated using methods developed by AHRQ and the Effective Health Care Program.[138] The strength of the evidence was evaluated based on four required domains: (1) risk of bias (do the studies for a given outcome or comparison have good internal validity); (2) consistency (the degree of similarity in the effect sizes (i.e., same direction of effect) of the included studies); (3) directness (reflecting a single, direct link between the intervention of interest and the outcome); and (4) precision (degree of certainty surrounding an effect estimate of a given outcome). The risk of bias, based on study design and conduct, is rated low, medium, or high. Consistency is rated consistent, inconsistent, or unknown/not applicable (e.g., a single study was evaluated). Directness can either be direct or indirect and precision is either precise or imprecise. A precise estimate is one that would yield a clinically meaningful conclusion.

The evidence is rated using high, moderate, low, and insufficient for grades. A high grade indicates that further research is very unlikely to change the confidence in the estimate of effect, meaning that the evidence is believed to reflect the true effect. A moderate grade denotes further research may change our confidence in the estimate of effect and may, in fact, change the estimate. A low grade indicates that further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate. Thus, there is low confidence that the evidence reflects the true effect. An insufficient grade indicates that the evidence is unavailable or does not permit a conclusion. An overall rating of high strength of evidence would imply that the included studies were RCTs with a low risk of bias and consistent, direct, and precise domains.

We modified this approach for diagnostic tests in the following manner. As previously stated, all of the studies that provided comparative evidence for differences between diagnostic tests were selected based on having ‘good’ protection against bias (internal validity). Furthermore, all of the comparative studies were rated as providing only indirect evidence because none presented evidence that the differences in sensitivity and/or specificity of the diagnostic tests would lead to any differences in patient outcomes. Indeed, studies that provide evidence that the observed differences would or would not be clinically meaningful were not found, nor were estimates of how much of a difference would be required to make a different clinical decision about the diagnosis. Thus, any differences in the overall grades of the strength of evidence for comparisons of the diagnostic tests are based on the consistency (direction and size) of the estimated differences in sensitivity and specificity and the precision (width of the estimated confidence intervals).

Applicability

Applicability of the treatment results, both standard and nonstandard adjuvant treatment, of this review are affected by the representativeness of the patient samples in the included studies, which are general adult inpatient populations. Applicability of diagnostic test results is limited by

the samples used in the analyses; to the extent that they were typical clinical samples derived from patients with suspected CDAD, they represent the typical patient population that was tested. However, the ability to explicitly state the applicability of such samples is dependent on the completeness of the study reporting on the characteristics of the patients/specimens that were selected for the study. Furthermore, the substantial heterogeneity between studies in estimates of sensitivity and specificity of many of the diagnostic tests being reviewed, and perhaps their differences, raises concerns about generalization of the results. The evidence tables in Appendix C identify reported details on the patient inclusion and exclusion criteria.

Data Synthesis

For key questions with trial data, we applied quantitative techniques to estimate a summary effect size for reported outcomes for which heterogeneity of interventions and outcomes measures was minimal. Qualitative narratives were provided for key questions for which heterogeneity of interventions or measured patient outcomes was too great, or for which available studies were observational. Results of the quantitative and qualitative analyses are compared to relevant published systematic reviews for consistency of findings. (See Appendix tables for details of systematic reviews.)

Data were analyzed in Review Manager 5.2.[139] Random effects models were used to generate pooled estimates of relative risks (RR) and weighted mean differences (WMD) with 95 percent confidence intervals. Statistical heterogeneity was summarized using the I^2 statistic (50 percent indicates moderate heterogeneity and 75 percent or greater indicates high heterogeneity).[140]

Key Question 1. We focused on the differences between test sensitivities and specificities, rather than the specific test sensitivities and specificities themselves. Thus, methods of meta-analysis typically used for clinical trials with binary endpoints were employed rather than methods typically used for sensitivities and specificities, such as diagnostic odds ratios. To be able to estimate the correlation between two tests that were applied to the same patients/stool specimens, hence calculate proper confidence intervals on the differences of the sensitivities and specificities of two tests, the results of each test for each individual are needed.[141] Many reports did not provide this information. Therefore, the estimated confidence intervals on the differences in sensitivities and specificities ignored the unknown correlation between test results. Ignoring the correlation most likely increased the estimated variances of the differences and the width of the confidence intervals depending on the direction and magnitude of the correlation between the estimates for the two tests.

Each study had two primary endpoints, difference in sensitivities and difference in specificities. Furthermore, some studies made multiple comparisons. Some adjustment for multiple endpoints and comparisons was made by calculating 99 percent confidence intervals on the differences.

Publication Bias

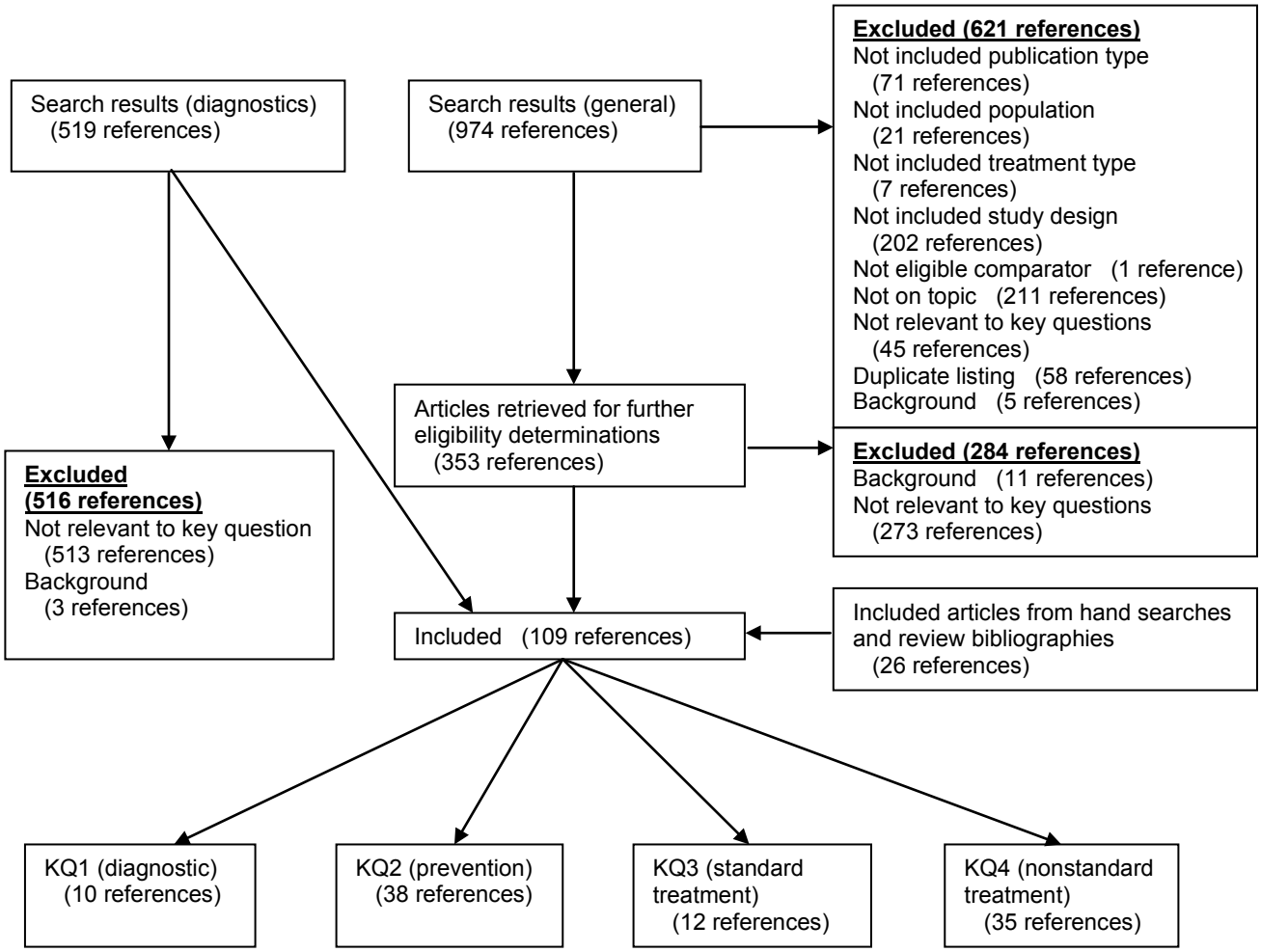
Grey literature was searched for relevant trials and other material to inform the likelihood of publication bias. Regulatory sources included Federal Drug Administration, Health Canada, Authorized Medicines for the European Union. Clinical trial registries accessed were ClinicalTrials.gov, Current Controlled Trials, Clinical Study Results, and World Health Organization's Clinical Trials. Grants and Federally funded research sources included NIH

RePORTER, a searchable database of federally funded biomedical research projects conducted at universities, hospitals, and other research institutions, and HRSPROJ, a database providing access to ongoing grants and contracts in health services research. Other sources searched were Hayes, Inc. Health Techology Assessment, New York Academny of Medicine's Greey Literature Index, Conference Papers Index, and Scopus.

Chapter 3. Results

The general search identified 974 citations from MEDLINE. Of these, 353 studies were pulled for full text screening. Of these 353 references, we included 69 RCT, systematic reviews, observational studies, plus an additional 26 articles obtained from hand searching and review article bibliographies. We excluded 905 articles. A supplemental search for diagnostics identified 519 citations from MEDLINE, of which 516 references were excluded. Figure 4 provides a literature flow diagram. A bibliography of the excluded articles, and their reasons for exclusion, is provided in Appendix D.

Figure 4. Reference flow diagram



Appendixes and evidence tables cited in this report are available at http://www.ahrq.gov/downloads/pub/evidence/pdf/XXX/XXX.pdf

Key Question 1. How do different methods for detection of toxigenic C. difficile compare in their sensitivity, specificity, and predictive values?

Search Results

We included 10 references that provided comparative data about diagnostic tests of interest. The studies were published from 2001 to 2010. Five studies were from the United States, two from the United Kingdom and one each from Spain, Belgium and Israel. Table 1 provides a summary of the available comparisons. Overall, these reports included data on seven named immunoassays for toxins A and B, one two-stage method where an immunoassay for glutamate dehydrogenase was combined with an immunoassay for toxins A and B, and two tests to detect gene fragments involved in the production of toxin B. Only three comparative studies included one of the recently FDA-approved toxin gene detection tests. Thus, the number and type of paired (within study) comparisons available for each diagnostic test varied considerably, and not all possible comparisons were available. Evidence summary tables, including study quality items, are available in Appendix C of this report (see Appendix Table C1).

Key Points

- Fifteen paired comparisons of seven commonly used immunoassays for toxins A and B provided low grade evidence that the test sensitivities do not differ. There was moderate grade evidence for no differences in test specificities for two comparisons and for a difference of 2 percent in one comparison. Otherwise, there was only low grade evidence for or against differences in test specificities. There was insufficient evidence of differences between all tests that were not directly compared.
- Nine comparisons of two different gene detection tests to toxin immunoassays or a 2-stage that first tested for GDH to provided only low grade evidence that the gene-based tests are substantially more sensitive. There was moderate evidence that the test specificities in one comparison did not differ. Otherwise, there was only low grade evidence for differences in either direction between test specificities. There was insufficient evidence of differences between all tests that were not directly compared.
- There was only low grade evidence that combining an immunoassay for GDH followed by an immunoassay of GDH positive specimens for toxins A and B reduced the percentage of false positives (increased the test specificity), but reduced the test sensitivity. There was insufficient evidence of differences between all tests that were not directly compared.
- There was insufficient evidence to determine whether any differences in sensitivity or specificity between diagnostic tests depend on patient or specimen characteristics or the clinical scenarios that lead to testing for toxigenic CDI.

Quality of the Comparative Studies

All studies used stool specimens from mostly inpatients that were submitted by clinicians to test for CDI. However, the clinical scenarios that prompted the clinicians to test for CDI, such as

the nature of the patient's diarrhea, or exposure to antibiotics, were not described in most reports. Six of the ten studies that provided data mentioned that the stool samples were liquid, unformed, or diarrhea, whereas the other reports did not clearly describe the consistency of the stool specimens. Four of the studies included more than one specimen from some patients, and two studies only reported the total number of stool specimens and not the number of patients. Four studies selected stool samples based on previous diagnostic test results to enhance the percentage of positive tests in their sample, and one included a facility with a recent outbreak of CDAD. Thus, the reviewed reports were somewhat deficient in reporting pertinent information about patient selection criteria and the spectrum of patients/specimens included the comparisons (Appendix Table C2).

Differences within studies in the timing and handling of specimens for the different tests being compared was not a major issue in the reviewed studies. Verification using the reference standard was applied consistently to all stool specimens. However, the same reference standard was not used in all studies. Four of the 10 studies used a cell cytotoxicity test as the reference, three used a cell cytotoxicity test in conjunction with toxigenic culture, two used toxigenic culture alone, and one used multiple immunoassays for toxins A and B in conjunction with toxigenic culture, and one used an in-house gene detection test. None of the reference methods that were used are true gold standards (a gold standard has not been established) in that they are not 100 percent sensitive or specific for true toxigenic CDI and their accuracies are not all the same. Within each study, the diagnostic tests were carried out independently of each other although the reports usually did not state that each test was interpreted without knowledge of other results. Only one report explicitly stated that all diagnostic tests being compared including the reference test were conducted in a blinded manner. Sometimes the independence of the tests could be inferred from their sequence and the time needed to get results.

The handling of indeterminate test results presents problems when comparing the sensitivity and specificity of diagnostic tests. Some investigators repeated indeterminate tests and used the result of the second test as recommended although some repeated tests were also indeterminate. Some assumed indeterminate results were negative and thereby could have inflated the number of false negatives. Some comparisons excluded indeterminate results, thus the varying number of indeterminate tests did not count for or against a test. However, differences in the number of interdeterminate results produced by different tests resulted in some differences in the stool specimens being used to compare the tests. Other types of subject or specimen withdrawal were not an issue in the studies that were reviewed.

Detailed Analysis

Comparisons of immunoassays for toxins A and B. As summarized in Table 2, none of the seven immunoassays for toxins A and B was compared to all others. When more than one study compared the same two immunoassays, the heterogeneity in the differences in sensitivity was not significant. None of the eight pooled comparisons based on two to four studies indicated that any of the immunoassays were more sensitive than another. The pooled estimates of the differences (99 percent confidence interval) in test sensitivities were 0±6 percent, 1±7 percent, 3±6 percent, 3±7 percent, -1±10 percent, 3±8 percent, 6±12 percent, and 1±9 percent. The confidence intervals for single-study estimates of differences in sensitivity were wide. Thus, the available data often could not rule out substantial differences in sensitivities.

There was some significant heterogeneity in the corresponding estimates of differences in false positives (1 – specificity) for two of the comparisons between immunoassays for toxins A and B. Ignoring the heterogeneity, the differences (99 percent confidence interval) in false positives were 0±2 percent, 0±1 percent, 2±1 percent, 0±1 percent, -3±3 percent, -1±10 percent, -6±14 percent and 3±2 percent. Thus, the available data often ruled out differences in false positives of only a few percent. One study that compared several immunoassays found some differences in the false positives of approximately 6 percent.

Gene detection tests versus immunoassays for toxins A and B. As summarized in Table 3, two studies compared the same test to detect genes related to toxin B production to an immunoassay for toxins A and B.[25,30] There was significant heterogeneity between the two that estimated differences in sensitivities for one comparison; however, both studies suggested the gene-based test was more sensitive than the immunoassay. The pooled estimate of the difference in sensitivities was 17 percent in favor of the gene based test with a 99 percent confidence interval of from 3 to 37 percent. There was no heterogeneity in the corresponding estimated differences in false positive percentages of these tests. The pooled estimate of the differences in the false positives was 0 percent with a 99 percent confidence interval of from 1 percent to 1 percent.

One of these studies also provided pair-wise comparisons of the gene detection test to six other immunoassays for toxins A and B.[25] The sensitivity of the gene detection test was consistently better, although the point difference ranged widely from 3 percent to16 percent and the confidence intervals didn't always exclude a difference of zero. Furthermore, the false positives for the gene-based test were approximately 3 percent greater compared to four of the immunoassays for toxins A and B.

A third study indicated that a different toxin B gene detection test was much more sensitive than one of the immunoassays for toxins A and B without a corresponding increase in the percentage of false positives.[33] However, the sensitivity of the immunoassay was remarkably low (only 58 percent) in this study for unknown reasons.

The sensitivities of the two gene detection tests in the three studies ranged from 89 percent to 94 percent. In contrast, the sensitivities of the immunoassays for toxins A and B were much more variable ranging from 58 percent to 86 percent. The methodological differences between studies, including use of different reference tests, might have affected the toxin immunoassays more than the gene detection tests.

Immunoassays for toxins A and B versus combined tests for GDH and toxins. A small single study that compared a different test for GDH combined with an immunoassay for toxins A and B to use of the immunoassay for toxins A and B by itself did not find a difference in sensitivity although the confidence intervals were very imprecise, 3 percent (99 percent confidence interval -24 percent to 18 percent) but did indicate the combined test had fewer false positives (-4 percent; 99 percent confidence interval -7 percent to 0 percent).[33]

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Table 1. Summary of diagnostic comparisons in included studies

	Premier Toxin A&B, Meridian	Tox A/B II, TechLab	Tox A/B QUIK CHEK, TechLab	ImmunoCard A&B, Meridian	Xpect Toxin A/B, Remel	ProSpecT Toxin A/B, Remel	C. diff Tox A/B, VIDAS	Combination Test*	Tox A/B TechLab
A and B Toxin Immunoassays									
Premier Toxin A&B, Meridian	******	4 studies	1 study	3 studies	2 studies	2 studies	1 study		
Tox A/B II, TechLab		*******	2 studies	2 studies					
Tox A/B QUIK CHEK, TechLab			********	4 studies	3 studies	1 study	1 study		
ImmunoCard A&B, Meridian				********					
Xpect Toxin A/B, Remel					******	1 study	1 study		
ProSpecT Toxin A/B, Remel						******	1 study		
C. diff Tox A/B, VIDAS							******		
Gene Detection Tests									
GeneOhm, Becton Dickinson	1 studies	2 studies	1 study	1 study	1 study	1 study	1 study		
GeneXpert, Cepheid	1 study							1 study	
Combination Test									
CHEK-60 (GDH), TechLab; then if positive Premier Toxin A&B, Meridian	1 study								

* CHEK-60 (GDH), TechLab; then if positive Premier Toxin A&B, Meridian

Table 2. Comparisons of immunoassays for toxins A and B

	Sensitivity (% True Positives)			% False Positives (1 – Specificity)		
	Toxin Immunoassay X	Toxin Immunoassay Y	% Difference	Toxin Immunoassay X	Toxin Immunoassay Y	% Difference
Eastwood 2009[25]	Premier Toxin A&B,	Tox A/B II,	1 (-12 to 14)†	Premier Toxin A&B,	Tox A/B II,	-1 (-4 to 1)
Musher 2007[26]	Meridian	TechLab	0 (-9 to 9)	Meridian	TechLab	-6 (-19 to 6)
Turgeon 2003[31]	101/125 (80.8%)	100/125 (80.0%)	-4 (-20 to 12)	12/475 (2.5%)	19/475 (4.0%)	0 (-1 to 1)
O'Connor 2001[29]	52/54 (96.3%)	52/54 (96.3%)	2 (-17 to 20)	5/77 (6.5%)	10/77 (13.0%)	0 (-3 to 3)
Pooled Estimate	74/101 (73.3%)	78/101 (77.2%)	0 (-7 to 6)	8/898 (0.9%)	5/902 (0.6%)	0 (-3 to 2)
heterogeneity‡	50/61 (82.0%)	49/61 (80.3%)	p=0.92;I^2=0%	1/139 (0.7%)	1/139 (0.7%)	p=0.06;I^2=60%
Eastwood 2009[25]	Tox A/B QUIK	ImmunoCard A&B,	0 (-15 to 14)	Tox A/B QUIK	ImmunoCard A&B,	0 (-1 to 1)
Alcala 2008 [24]	CHEK, TechLab	Meridian	-12 (-29 to 6)	CHEK, TechLab	Meridian	0 (-5 to 4)
Deyi 2008 [32]	93/125 (74.4%)	86/115 (74.8%)	4 (-14 to 23)	3/473 (0.6%)	2/444 (0.4%)	0 (-3 to 3)
Samra 2008[27]	56/102 (54.9%)	68/102 (66.7%)	0 (-8 to 8)	12/265 (4.5%)	13/265 (4.9%)	0 (-6 to 6)
Pooled Estimate	22/23 (95.7%)	21/23 (91.3%)	-1 (-9 to 6)	0/77 (0%)	0/77 (0%)	0 (-1 to 1)
heterogeneity	89/94 (94.7%)	89/94 (94.7%)	p=0.26;I^2=25%	3/106 (2.8%)	3/106 (2.8%)	p=0.98;I^2=0%
Eastwood 2009[25]	Premier Toxin A&B,	ImmunoCard A&B,	6 (-8 to 20)	Premier Toxin A&B,	ImmunoCard A&B,	2 (0 to 4)
Sloan 2008	Meridian	Meridian	0 (-27 to 27)	Meridian	Meridian	1 (-3 to 4)
Musher 2007[26]	101/125 (80.8%)	86/115 (74.8%)	3 (-4 to 9)	12/475 (2.5%)	2/444 (0.4%)	2 (-1 to 4)
Pooled Estimate	21/44 (47.7%)	21/44 (47.7%)	3 (-3 to 9)	3/156 (1.9%)	2/156 (1.3%)	2 (0 to 3)
heterogeneity	75/76 (98.7%)	73/76 (96.1%)	p=0.77;I^2=0%	10/370 (2.7%)	4/370 (1.1%)	p=0.67;I^2=0%
Eastwood 2009[25]	Tox A/B QUIK	Xpect Toxin A/B,	1 (-14 to 15)	Tox A/B QUIK	Xpect Toxin A/B,	0 (-1 to 1)
Alcala 2008 [24]	CHEK, TechLab	Remel	6 (-12 to 24)	CHEK, TechLab	Remel	0 (-4 to 5)
Deyi 2008 [32]	93/125 (74.4%)	86/117 (73.5%)	4 (-14 to 23)	3/473 (0.6%)	3/475 (0.6%)	0 (-3 to 3)
Pooled Estimate	56/102 (54.9%)	50/102 (49.0%)	3 (-6 to 13)	12/265 (4.5%)	11/265 (4.2%)	0 (-1 to 1)
heterogeneity	22/23 (95.7%)	21/23 (91.3%)	p=0.84;I^2=0%	0/77 (0%)	0/77 (0%)	p=0.96;I^2=0%
Eastwood 2009[25]	Tox A/B QUIK	Tox A/B II,	-6 (-19 to 8)	Tox A/B QUIK	Tox A/B II,	-3 (-6 to -1)
Samra 2008[27]	CHEK, TechLab	TechLab	1 (-8 to 10)	CHEK, TechLab	TechLab	-3 (-10 to 4)
Pooled Estimate	93/125 (74.4%)	100/125 (80.0%)	-1 (-11 to 8)	3/473 (0.6%)	19/475 (4.0%)	-3 (-6 to -1)
heterogeneity	89/94 (94.7%)	88/94 (93.6%)	p=0.22;I^2=35%	3/106 (2.8%)	6/106 (5.7%)	p=0.85;I^2=0%
Eastwood 2009[25]	Premier Toxin A&B,	ProSpecT Toxin	-1 (-14 to 12)	Premier Toxin A&B,	ProSpecT Toxin	-4 (-8 to -1)
Musher 2007[26]	Meridian	A/B, Remel	6 (-7 to 8)	Meridian	A/B, Remel	4 (-5 to 13)
Pooled Estimate	101/125 (80.8%)	102/125 (81.6%)	3 (-6 to 11)	12/475 (2.5%)	32/475 (6.7%)	-1 (-11 to 10)
heterogeneity	52/54 (96.3%)	49/54 (90.7%)	p=0.31;I^2=4%	5/77 (6.5%)	2/77 (2.6%)	p=0.02;I^2=80%
Eastwood 2009[25]	Premier Toxin A&B,	Xpect Toxin A/B,	7 (-7 to 21)	Premier Toxin A&B,	Xpect Toxin A/B,	2 (0 to 4)
Sloan 2008[28]	Meridian	Remel	0 (-27 to 27)	Meridian	Remel	-14 (-22 to -6)
Pooled Estimate	101/125 (80.8%)	86/117 (73.5%)	6 (-7 to 18)	12/475 (2.5%)	3/475 (0.6%)	- 6 (-32 to 20)
heterogeneity	21/44 (47.7%)	21/44 (47.7%)	p=0.54;I^2=0%	3/156 (1.9%)	25/156 (16.0%)	p=0.0001;I^2=97%
Eastwood 2009[25]	Tox A/B II, TechLab	ImmunoCard A&B,	5 (-9 to 19)	Tox A/B II, TechLab	ImmunoCard A&B,	4 (1 to 6)
Samra 2008[27]	100/125 (80.0%)	Meridian	-1 (-10 to 8)	19/475 (4.0%)	Meridian	3 (-4 to 10)
Pooled Estimate	88/94 (93.6%)	86/115 (74.8%)	1 (-8 to 10)	6/106 (5.7%)	2/444 (0.4%)	3 (1 to 6)
heterogeneity		89/94 (94.7%)	p=0.25;I^2=24%		3/106 (2.8%)	p=0.80;I^2=0%

Table 2. Comparisons of immunoassays for toxins A and B (continued)

	Sensitivity (% True Positives)			% False Positives (1 – Specificity)		
	Toxin Immunoassay X	Toxin Immunoassay Y	% Difference	Toxin Immunoassay X	Toxin Immunoassay Y	% Difference
Eastwood 2009[25]	Premier Toxin A&B, Meridian 101/125 (80.8%)	C. diff Tox A/B, VIDAS 100/116 (86.2%)	-5 (-18 to 7)	Premier Toxin A&B, Meridian 12/475 (2.5%)	C. diff Tox A/B, VIDAS 2/464 (0.4%)	2 (0 to 4)
Eastwood 2009[25]	Premier Toxin A&B, Meridian 101/125 (80.8%)	Tox A/B QUIK CHEK, TechLab 93/125 (74.4%)	6 (-7 to 20)	Premier Toxin A&B, Meridian 12/475 (2.5%)	Tox A/B QUIK CHEK, TechLab 3/473 (0.6%)	2 (0 to 4)
Eastwood 2009[25]	Tox A/B QUIK CHEK, TechLab 93/125 (74.4%)	ProSpecT Toxin A/B, Remel 102/125 (81.6%)	-7 (-21 to 6)	Tox A/B QUIK CHEK, TechLab 3/473 (0.6%)	ProSpecT Toxin A/B, Remel 32/475 (6.7%)	-6 (-9 to -3)
Eastwood 2009[25]	Tox A/B QUIK CHEK, TechLab 93/125 (74.4%)	C. diff Tox A/B, VIDAS 100/116 (86.2%)	-12 (-25 to 1)	Tox A/B QUIK CHEK, TechLab 3/473 (0.6%)	C. diff Tox A/B, VIDAS 2/464 (0.4%)	0 (-1 to 1)
Eastwood 2009[25]	Xpect Toxin A/B, Remel 86/117 (73.5%)	ProSpecT Toxin A/B, Remel 102/125 (81.6%)	-8 (-22 to 6)	Xpect Toxin A/B, Remel 3/475 (0.6%)	ProSpecT Toxin A/B, Remel 32/475 (6.7%)	-6 (-9 to -3)
Eastwood 2009[25]	Xpect Toxin A/B, Remel 86/117 (73.5%)	C. diff Tox A/B, VIDAS 100/116 (86.2%)	-13 (-26 to 1)	Xpect Toxin A/B, Remel 3/475 (0.6%)	C. diff Tox A/B, VIDAS 2/464 (0.4%)	0 (-1 to 1)
Eastwood 2009[25]	ProSpecT Toxin A/B, Remel 102/125 (81.6%)	C. diff Tox A/B, VIDAS 100/116 (86.2%)	-5 (-17 to 8)	ProSpecT Toxin A/B, Remel 32/475 (6.7%)	C. diff Tox A/B, VIDAS 2/464 (0.4%)	6 (3 to 9)

† Values in parentheses are 99% confidence intervals for the difference between tests conservatively assuming statistical independence between the paired tests. Bold numbers indicate the 99% confidence interval excludes a difference of zero. ‡The p-value is a chi-square test for nonrandom variation in the differences between studies, and I^2 is the proportion of the total variance in the estimated differences that reflects nonrandom variation.

Table 3. Toxin gene tests compared to immunoassays

	Sensitivity (% True Positives)			% False Positives (1 – Specificity)		
	Toxin Gene Test	Toxin Immunoassay	% Difference	Toxin Gene Test	Toxin Immunoassay	% Difference
Kvach 2010 [30]	GeneOhm, Becton Dickinson 96/105 (91%)	Tox A/B II, TechLab 70/105 (67%)	25 (11 to 39)†	GeneOhm, Becton Dickinson 0/295 (0%)	Tox A/B II, TechLab 1/295 (0.3%)	0 (-4 to 3)
Eastwood 2009[25]	92/103 (89%)	100/125 (80%)	9 (-3 to 21)	16/449 (3.6%)	19/475 (4.0%)	0 (-2 to 1)
Pooled Estimate			17 (-3 to 37)			0 (-1 to 1)
heterogeneity‡			p=0.03;I^2=79%			p=0.90;I^2=0%
Eastwood 2009[25]	GeneOhm, Becton Dickinson 92/103 (89%)	Premier Toxin A&B, Meridian 101/125 (81%)	9 (-3 to 21)	GeneOhm, Becton Dickinson 16/449 (3.6%)	Premier Toxin A&B, Meridian 12/475 (2.5%)	1 (-2 to 4)
Eastwood 2009[25]	GeneOhm, Becton Dickinson 92/103 (89%)	ImmunoCard A&B, Meridian 86/115 (75%)	15 (1 to 28)	GeneOhm, Becton Dickinson 16/449 (3.6%)	ImmunoCard Toxin A&B, Meridian 2/444 (0.4%)	3 (1 to 6)
Eastwood 2009[25]	GeneOhm, Becton Dickinson 92/103 (89%)	Tox A/B QUIK CHEK, TechLab 93/125 (74%)	15 (2 to 28)	GeneOhm, Becton Dickinson 16/449 (3.6%)	Tox A/B QUIK CHEK, TechLab 3/473 (0.6%)	3 (0 to 5)
Eastwood 2009[25]	GeneOhm, Becton Dickinson 92/103 (89%)	ProSpecT Toxin A/B, Remel 102/125 (82%)	8 (-4 to 20)	GeneOhm, Becton Dickinson 16/449 (3.6%)	ProSpecT Toxin A/B, Remel 32/475 (6.7%)	-3 (-7 to 1)
Eastwood 2009[25]	GeneOhm, Becton Dickinson 92/103 (89%)	Xpect Toxin A/B, Remel 86/117 (74%)	16 (3 to 29)	GeneOhm, Becton Dickinson 16/449 (3.6%)	Xpect Toxin A/B, Remel 3/475 (0.6%)	3 (0 to 5)
Eastwood 2009[25]	GeneOhm, Becton Dickinson 92/103 (89%)	C. diff Tox A/B, VIDAS 100/116 (86%)	3 (-8 to 14)	GeneOhm, Becton Dickinson 16/449 (3.6%)	C. diff Tox A/B, VIDAS 2/464 (0.4%)	3 (1 to 6)
Novak-Weekley, 2010[33]	GeneXpert, Cepheid 68/72 (94%)	Premier Toxin A&B, Meridian 42/72 (58%)	36 (20 to 53)	GeneXpert, Cepheid 13/356 (3.7%)	Premier Toxin A&B, Meridian 19/360 (5.3%)	-2 (-6 to 2)
Novak-Weekley, 2010[33]	GeneXpert, Cepheid 68/72 (94%)	CHEK-60 (GDH), TechLab; if + then Toxin A&B, Meridian 40/72 (56%)	39 (22 to 55)	GeneXpert, Cepheid 13/356 (3.7%)	CHEK-60 (GDH), TechLab; if + then Toxin A&B, Meridian 6/360 (1.7%)	2 (-1 to 5)

† Values in parentheses are 99% confidence intervals for the difference between tests conservatively assuming statistical independence between the paired tests. Bold numbers indicate the confidence interval excludes a difference of zero. ‡The p-value is a chi-square test for nonrandom variation in the differences between studies, and I^2 is the proportion of the total variance in the estimated differences that reflects nonrandom variation.

Key Question 2. What are effective prevention strategies?

Search Results

We found one Cochrane review, [34] four studies on antibiotic prescribing restrictions,[35-38], 10 on single preventive practices aimed at transmission interruption,[39-48] and 10 studies that bundled multiple practices into a prevention strategy.[49-58] Only two trials were controlled trials;[39,42]one was an interrupted time series study,[35,59]and the remaining studies were before/after designs.[36-38,40,41,43-48,59] The included studies are provided in Table 4.

Twelve studies examining risk factors met the inclusion criteria and updated the period following a systematic review.[14] (Appendix Table C3). Six studies were conducted in the United States,[98,142-146] two in the United Kingdom,[147,148] two in Israel,[99,149] one in Spain,[150] and one study was based in the United States and Canada.[151] The average CDAD patient sample was 72 patients, with a range of 28 to 155. Studies varied in the degree to which the investigators verified that positive tests reflected disease.

Key Points

- Overall, the evidence available to link prevention strategies to clinically important outcomes, such as CDAD incidence, is of low quality and is not extensive.
- Four observations studies, and one Cochrane Review, found prescribing practice interventions decreasing the use of high risk antimicrobials are associated with decreased CDAD incidence. Prescribing practices were also used in multicomponent interventions credited with reducing CDAD incidence; however, it is difficult to isolate the specific effects of the prescribing practices.
- One controlled trial found glove use significantly reduced CDAD incidence.
- Three observational studies, including two controlled, found disposable thermometer use is likely to reduce CDAD incidence.
- No study examined the effect of handwashing, rather than alcohol gels, on CDAD incidence. Four observational studies found use of alcohol gels, presumably in the presence of protocols requiring handwashing in the presence of CDAD or visible soiling, did not increase CDAD incidence.
- Three studies provide low evidence that disinfection with a chemical compound that kills *C. difficile spores* in the hospital environment prevents CDAD, at least in epidemic or hyperendemic settings. Seven studies included disinfection in multicomponent interventions. Disinfection agents examined included hypochlorite solution, hydrogen peroxide, aldehydes, and detergent.
- Ten time series/before-after studies have examined bundled multiple interventions using before/after study designs. Data is insufficient to draw conclusions.
- Risk factors for developing CDAD include antibiotic use, substantial chronic illness, hospitalization in an ICU, and age. There are conflicting data about drugs used for acid suppression.
- No data on patient harms or harms to hospital staff due to preventive interventions were reported.

- No studies assessed the sustainability of a prevention program beyond an intervention period.

Quality of the Studies

Overall, the quality of the evaluated studies was considered low. In the Cochrane Review[34] focusing on improving antibiotic prescribing practices, the evidence from one article[152] was judged to be of "good" quality and evidence from the others was considered "weak." The evidence for the 10 single preventive practices aimed at transmission interruption was low because they predominately used before/after design, and were done in response to epidemic or hyperendemic conditions. In particular, there is insufficient evidence that **hand washing** is associated with reduced CDAD incidence as no study assessed this intervention. Of the four studies assessing alcohol based rubs or gels, only one had concurrent controls. Thirteen studies examined if **disinfections** were all before/after studies, generally done in response to epidemics.

For the 10 articles that described multiple component preventive interventions, none had concurrent controls or was blinded, and there was considerable variability in the types of interventions so that pooling could not be done. In addition, it was indeterminable to attribute decreases in CDAD incidence to any single intervention in all of these studies.

Detailed Analysis

Due to the low quality studies, we provide a qualitative narrative of the evidence for prevention practice interventions.

Antibiotic use. The five studies summarized in the Cochrane Review[34], and the additional four individual studies here,[35-38] found that changes in antimicrobial education, policies, or formularies, which result in decreasing use of high risk antimicrobials, are associated with decreased CDAD incidence. It was not possible to clearly isolate the impact of the antibiotic-related interventions in the studies examining multiple interventions.[51-53,55,56] In the individual studies, which were usually done in response to outbreaks, interventions in addition to those aimed at antibiotic use may have been done but not reported. The interventions and antibiotics targeted for reduction differed among the various studies.

The Cochrane review[34] determined the impact of interventions to improve antibiotic prescribing practices for hospital inpatients on CDAD incidence. The authors found that four interventions were associated with significant reductions in CDAD incidence[55,152-154] and that one was associated with a nonsignificant trend towards a reduction.[54]

A prospective controlled interrupted time series[35] of an antibiotic improvement intervention on three acute medical wards for elderly people with 21 month pre-defined pre- and post-intervention periods, evaluated a 'narrow-spectrum' antibiotic policy (reinforced by an established program of audit and feedback of antibiotic usage and CDI rates). The program targeted broad-spectrum antibiotics (cephalosporins and amoxicillin/clavulanate) for reduction and narrow-spectrum antibiotics (benzyl penicillin, amoxicillin. and trimethoprim) for increase. CDAD rates decreased significantly with incidence rate ratios of 0.35 (95 percent CI 0.17 – 0.73). MRSA incidence, the control, did not change significantly.

The effect of a new antibiotic policy favoring piperacillin-tazobactam over cefotaxime on the long-term incidence of CDAD and antibiotic utilization in a large elderly medicine unit was studied in a before-after observational study.[36] Restrictions were associated with reduced

cefotaxime use and reduced CDAD incidence. Subsequently, the piperacillin-tazobactam became unavailable at the end of 2001. Cefotaxime use and CDAD incidence rates increased during 2002.

In a geriatric department of a university hospital, antimicrobial recommendations for treatment of several common infectious diseases were changed from broad-spectrum cephalosporins to other drugs thought to be less likely to induce CDAD.[37] Investigators changed department policy to reflect these recommendations, educated providers, monitored antibiotic use, and gave periodic feedback to providers. Cephalosporin use dropped and the relative risk of CDAD decreased to 0.31 (95 percent CI 0.93 to 0.10) compared with usage before the policy change.

In a geriatric department of another university hospital, broad spectrum cephalosporin use was restricted due to an increase in CDAD incidence.[38] In the following year, cephalosporin use decreased 92 percent, and CDAD incidence decreased 50 percent from the previous year incidence. CDAD incidence did not change in other hospital departments.

Measures to reduce transmission.

Gloves. One controlled trial examined the use of gloves to prevent *C. difficile* transmission, with CDAD incidence monitored by active surveillance.[39] An intensive education campaign on two wards that urged personnel to use gloves when handling body substances, and gloves were made easily available to personnel working with patients. Two other wards with no education campaign served as control wards, and gloves on these wards were stocked in supply rooms. Incidence of CDAD decreased significantly from 7.7 cases/1,000 patient discharges during the six months before intervention to 1.5/1,000 during the six months of intervention on the intervention wards. No significant change in CDAD incidence was observed on the control wards. Asymptomatic *C. difficile* carriage also decreased significantly on the intervention wards but not on the control wards. The cost of 61,500 gloves (4,505 gloves/100 patients) used was $2,768 for the glove-using wards, compared with $1,895 (42,100 gloves; 3,532 gloves/100 patients) on the control wards.

Disposable thermometers. Three studies, one randomized crossover design,[42]and two before after studies without concurrent controls,[40,41] have shown that use of disposable thermometers prevent CDAD. In one hospital with an increased CDAD incidence, 21 percent of electronic rectal thermometer handles were contaminated with *C difficile*.[40] Efforts to reinforce infection control practices already in place, but CDAD incidence remained elevated. A before/after trial was conducted in that hospital and a chronic care facility to determine if use of disposable thermometers instead of multiple use electronic rectal thermometers would reduce the CDAD incidence. Surveillance for CDAD was active, but toxin was detected with a latex agglutination test. During the 6-month post-intervention period, the CDAD incidence decreased from 2.71/1,000 patient days to 1.76/1,000 patient days in the acute hospital and from 0.41/1,000 patient days to 0.11/1,000 patient days in the skilled nursing facility. The harms associated with use of disposable thermometers were costs for purchase of disposable thermometers and the need to dispose of these thermometers. In these institutions, annual outlays increased from $7,731 to $14,055. These costs were offset by the need to purchase fewer electronic thermometers and to sterilize them periodically and by decreased costs of treating CDAD cases.

In a later report, the same group reported that the rate of *C. difficile* infections increased in the period 1991 to 1993, although it was unclear how many patients had symptoms of disease with *C. difficile*.[41] One ward used disposable tympanic membrane thermometers instead of disposal oral or rectal thermometers. Different interventions were implemented in two other

wards. Regression analysis determined that the *C. difficile* infection rate decreased 40 percent (RR, 0.59; 95 percent CI, 0.47-0.67).

A randomized, controlled crossover study compared the use of disposable thermometers with electronic thermometers to prevent nosocomial CDAD.[42] Twenty hospital wards were randomly assigned to disposable thermometers or electronic thermometers for 6 months, and then the assignments were reversed for 5 months. CDAD rates were reduced 44 percent (P=0.026, 95 percent CI, 0. 21-0.93) with disposable thermometers compared to electronic thermometers. Rates of nosocomial diarrhea or nosocomial infections did not differ significantly between the two groups. A cost analysis estimated that the hospital using disposable thermometers would need to spend an additional $5,926 to prevent a single CDAD case. It was estimated that a CDAD case resulted in from $2,000 to $6,000 in excess costs.

Hand washing. No study addressed whether hand washing was associated with reduced CDAD incidence. Many institutions encourage the use of alcohol based rubs or gels for hand hygiene unless hands are grossly soiled or unless a health care worker has had potential contact with *C. difficile* either from patient contact or environmental contamination. Complicated recommendations are difficult to remember and implement, and one concern has been that health care workers will use alcohol based rubs or gels in circumstances where hand washing is preferred. Since alcohol does not kill *C. difficile* spores, this may result in the spread of *C. difficile* within hospital environments.

Four studies have addressed this concern. One 2-year, prospective, controlled, cross-over trial compared alcohol-based hand gel provided in addition to hand soap containing the antimicrobial 0.3 percent chloroxylenol with antimicrobial soap alone in two intensive care units.[43] In units using adjuvant alcohol-based gel, there was a significant, sustained improvement in the rate of hand hygiene adherence but no detectable change in the incidence of healthcare-associated CDAD (diagnosis determined by clinicians).[43] Employees still had access to soap and water when their hands were soiled or when they were caring for a patient with *C. difficile,* and if workers used soap and water in these circumstances, it would have decreased the likelihood that differences in CDAD rates would be detected.

The second study used a before-after design.[44] Hospital employees were encouraged to wash hands with the antimicrobial 0.3 percent triclosan in the first three year period, and an alcohol-based hand rub with 62.5 percent ethyl was placed in dispensers in inpatient and outpatient clinic rooms in the next three years. There was a 21 percent decrease in new, nosocomially acquired MRSA isolates and a 41 percent decrease in VRE isolates, but the incidence of new CDAD cases remained similar(diagnosis determined by clinicians/toxin A assay).[44]

The secondary objective of a retrospective time series analysis was done to determine the relationship between use of alcohol-based hand rub and antibiotic consumption on the incidence of CDAD.[45] CDAD incidence was determined retrospectively from records of patients put in isolation for CDAD. Multivariable time series analyses showed no association between alcohol-based hand rub and CDAD incidence. Macrolide and third-generation cephalosporin use was associated with increased CDAD incidence after lag times of 1 to 3 months.

A retrospective, interventional time-series analysis was used to determine the effects of two interventions on CDAD incidence.[59] The interventions were promotional campaigns to encourage use of alcohol-based hand rub for hand hygiene. Time series analysis was done with autoregressive integrated moving average models. There was no association between alcohol-based hand rub and CDAD incidence.

Disinfection. Three studies examined if disinfection reduces the incidence of CDAD as a single component intervention,[46-48] and seven studies included disinfection in multicomponent interventions.[49,50,52,53,56,58,60] Disinfection agents examined included hypochlorite solution, hydrogen peroxide, aldehydes, and detergent.

Two studies examined **hypochlorite solution** as a single intervention. One before-after intervention investigated whether cleaning patient rooms with a positive test for *C. difficile* toxin with unbuffered with 1:10 hypochlorite solution reduced the incidence of CDAD in three patients units.[46] Before the intervention, patient rooms were cleaned with quaternary ammonium. In one housing bone marrow transplant patients and having the greatest rate before the intervention, the CDAD incidence rate decreased significantly, from 8.6 to 3.3 cases per 1,000 patient-days (hazard ratio, 0.37; 95 percent CI, 0.19–0.74) after hypochlorite was used to clean rooms. In the other two with lesser rates before the intervention, there was no significant change. In response to a subsequent outbreak of VRE infections, the hospital used quaternary ammonium solution for all patient room disinfection. The incidence of VRE infection decreased, but the CDAD incidence rate increased. Hypochlorite disinfection was re-instituted and the CDAD incidence rate subsequently decreased. A followup report documented subsequent increases in incidence and further interventions to control CDAD.

An epidemiological investigation of an outbreak of CDAD occurring in a single ward of a Michigan hospital documented nosocomial acquisition from the environment.[47] After use of unbuffered hypochlorite to disinfect wards, contamination decreased and the outbreak ended. Subsequently, it was shown that phosphate-buffered hypochlorite was even more effective for disinfection.

Hypochlorite was used in various ways in conjunction with other interventions to prevent CDAD in seven studies (multiple intervention table part B).[49,50,52,53,56,58,60] The effect of the hypochlorite disinfection cannot be isolated from the other intervention components.

Two studies were conducted to evaluate whether disinfection with **hydrogen peroxide** as part of multiple component interventions reduces CDAD incidence.[50,56] In the first study, an abrupt increase in nosocomial CDAD (defined as diarrhea with a positive toxin test) incidence led to multiple interventions in attempts to control the outbreak. Surveillance was based on laboratory and patient medical records.[50] A liquid vapor hydrogen peroxide decontamination system was used to decontaminate five high incidence wards of *C. difficile* organisms following a slight decrease in nosocomial CDAD incidence without concurrent controls.[50] Liquid vapor hydrogen peroxide was then used to decontaminate patient rooms vacated by patients with CDAD throughout the hospital on an ongoing basis. Nosocomial CDAD incidence continued to decrease and remained at lower levels. Quality of the diagnosis and surveillance system was good. No harms to hospital personnel, patients, or equipment were observed. The authors noted that the area to be decontaminated must be appropriately sealed, hydrogen peroxide levels outside the area being decontaminated must be closely monitored, and hydrogen peroxide concentrations within the decontaminated area must be reduced to less than 1 part per million before allowing patients or healthcare workers to re-enter. A subsequent study by the same investigators reported that hydrogen peroxide vapor disinfection was feasible in their hospital.[155] The peroxide vapor disinfection took 2 hours and 20 minutes to complete compared with 32 minutes for routine cleaning. The median cumulative times for all phases of cleaning and disinfection were 234 minutes (174–838) for peroxide vapor compared with 55 minutes (range 28–256) for conventional hypochlorite.

In the second study, 7 percent accelerated hydrogen peroxide was used for terminal disinfection of rooms of patients with CDAD and comprehensive ward disinfection with sodium hypochlorite was done when three or more nosocomial CDAD cases (defined as cases with positive toxin or with endoscopic or histological evidence of pseudo membranous colitis) remained elevated.[56] Within 4 months of the time infection prevention measures were implemented, the investigators also took several steps to reduce antibiotic use. Nosocomial CDAD incidence fell abruptly within 1 month of the changes in antibiotic use.

In one study using **aldehydes** as part of a multiple component intervention, a cluster of CDAD in a surgical ward led to a hospital-wide surveillance and control program.[48] Control interventions included terminal room disinfection with 0.04 percent formaldehyde and 0.03 percent glutaraldehyde in wards with a cluster of two or more nosocomial CDAD cases per month. During a 12-month period, the quarterly incidence of nosocomial CDAD remained unchanged. *C. difficile* spores were recovered from 36.7 percent surfaces of case patient rooms versus 6.7 percent in control rooms. Subsequently, more intensive control measures were evaluated, which included daily meticulous room disinfection for each sporadic nosocomial CDAD case. Surface disinfection reduced the contamination level four-fold ($p = 0.04$). In the following 12 months, the nosocomial CDAD incidence fell to 0.3/1,000 admission (protective efficacy 73 percent, 95 percent CI, 46-87 percent). Multiple interventions, including disinfection, were used to control the outbreak. The study provides low evidence that disinfection, in this case with aldehydes, might have had a role in terminating the outbreak.

These 10 studies provide low evidence that disinfection with a chemical compound that kills *C. difficile spores* in the hospital environment prevents CDAD, at least in epidemic or hyperendemic settings. Decreased CDAD incidence might have been from natural variation (regression to the mean) in some or all studies. As stated previously, disinfection was one of multiple interventions used to prevent CDAD in seven studies; it is difficult to impossible to know which intervention or combination of interventions might have led to reduced CDAD incidence.

Multiple component studies. Ten studies described the use of multiple preventive measures to control epidemic CDAD, or endemic CDAD which was felt to be excessive. Tables 5 and 6 list the categories of interventions in each of these articles. The number of interventions and the specific nature of any particular interventions varied widely. Studies employed between two and nine different types of interventions, including steps to optimize antimicrobial (six studies),[51-56] enhanced surveillance (two studies)[52,53] intensified staff education about infection prevention (three studies)[53,55,56] new isolation procedures (four studies),[52,54,56,57] and "enteric precautions" (two studies).[51,54] Two studies emphasized hand washing,[54,57] and one alcohol-based gel for hand disinfection.[53] Health care workers were required to wear gloves in three studies,[54,55,57] and requirement of gowns for patient contact were used in two studies.[54,57] Visitors were asked to comply with infection prevention procedures in one study.[57] New dedicated patient care equipment was purchased in two studies,[53,56] and in one of these, cleaning of dedicated patient equipment was intensified. Disposable rectal thermometers were used in one study.[56] Intensified environmental cleaning was implemented in six studies.[52-57] CDAD patient movement was restricted in two studies.[52,57]

Investigators often placed greater weight on one intervention over others because the timing of decreased CDAD incidence appeared to follow implementation of a particular intervention. However, the time it takes for many interventions to become adopted in health care settings and the variance expected in disease incidence led us to conclude that it was not possible to attribute

decreases in CDAD incidence to a single intervention in any of these studies. Natural fluctuations are such that all outbreaks diminish after variable periods of time so that assigning causality to individual or a collection of prevention measures is impossible. The evidence from these studies that any single intervention or combination of interventions prevents CDAD was low.

Harms. Harms, beyond cost, were not addressed in any study.

Risk Factors. Identified CDAD risk factors can provide clues to researchers and health care providers for where to target prevention strategies. One systematic review reviewed CDAD risk factor literature through 1997[14]and 12 risk factor studies that were published after the review. Bignardi's systematic review identified risk factors with "substantive" evidence: age, severity of underlying diseases, nonsurgical GI procedures, nasogastric tube, acid suppression medications, ICU, length of stay, duration of antibiotic course, and multiple antibiotics.[14] A further eight studies identified specific antibiotics or antibiotic classes with increased CDAD risk,[98,142,145-147,151,156] (Table 7), and four studies found that antibiotic use in general was associated with increased risk for CDAD.[98,142,148] Consistent with Bignardi's findings, the more recent literature also identified severe underlying disease as a risk factor in four studies,[99,143,144,148] and acid suppression in one,[99]

Sustainability. No studies addressed the sustainability of a prevention program.

Table 4. Prevention interventions

Author/Year Country	Study Design	Population	Intervention	Outcome	Findings	Quality Issues
Antibiotic Use						
Fowler, 2007[35] UK	Prospective interrupted Time Series	Acute medical wards, elderly	Switch from broad to narrow spectrum antibiotics	CDAD incidence	Incidence rate decreased (0.35, CI 0.17 – 0.73)	No concurrent controls; MRSA as control (?)
Davey, 2005[34] UK	Cochrane Review Five included studies	Hospital inpatients	Improve antibiotic prescribing practices	CDAD incidence	Four interventions were associated with significant reductions in CDAD	Only one study was judged to be of "good" quality
O'Connor, 2004[37]	Before/after	Geriatric unit N=17 cases in 683 patients	Change in antibiotic policy; education, monitoring, feedback	CDAD incidence	CDAD rate decreased significantly. Use of restricted antibiotic decreased. RR 0.31 (CI 0.93 – 0.10)	Retrospective
Wilcox, 2004[36]	Before/ after time series	Elderly Medicine Unit inpatients	Change in antibiotic policy	CDAD incidence	Use of restricted antibiotic decrease	No concurrent control
Ludlam, 1999[38] UK	Prospective before/after time series	Hospital N=4,284	Change in antibiotic policy	CDAD incidence	CDAD rate decreased 50%. Use of restricted antibiotic decreased 92%	Patients on wards were antibiotic policy was unchanged acted as controls
Transmission Interruption - Gloves						
Johnson, 1990[157] USA	Controlled trial		Education program to use gloves	CDAD incidence	CDAD incidence decreased from 7.7 cases/1,000 patient discharges to 1.5/1,000 discharges	Not randomized or blinded
Transmission Interruption – Disposable Thermometers						
Brooks, 1992[40] and 1998[41] USA	Time series (before/ after)	Hospital and long-term care	Single use thermometers	CDAD incidence	Decrease in incidence: acute care - from 2.71/1,000 patient days to 1.76/1000 Long term-care - from 0.41/1,000 patient days to 0.11/1,000 patient days	No concurrent controls
Jernigan, 1998[42] USA	Crossover RCT	Hospital patients admitted to 20 nursing units	Disposable thermometers versus electronic thermometers	Rate of nosocomial CDAD	CDAD rates were reduced 44 percent (95 percent confidence interval, 21-93) with	Two wards elected not to use disposable thermometers

Table 4. Prevention interventions (continued)

Author/Year Country	Study Design	Population	Intervention	Outcome	Findings	Quality Issues
					disposable thermometers compared to electronic thermometers. Rates of nosocomial diarrhea or nosocomial infections did not differ significantly between the two groups.	
Transmission Interruption – Hand washing						
Kaier, 2009[45] Germany	Before/after time series analysis	Tertiary care teaching hospital	Alcohol-based gel	CDAD incidence	No association between alcohol-based hand rub and CDAD incidence	Retrospective, no concurrent control
Vernaz, 2008[59] Switzerland	Before/after time series analysis	Primary and tertiary care teaching hospital	Promotional campaigns to encourage use of alcohol-based hand rub	CDAD incidence	No association between alcohol-based hand rub and CDAD incidence	Retrospective, no concurrent control
Rupp, 2008[43] USA	Controlled cross-over trial	Adult medical-surgical ICUs	Alcohol-based gel	CDAD incidence	Use of gel adherence rates increased from 37% to 68%. No change in CDAD rates	Not blinded
Gordin, 2005[44] USA	Before/after	Hospital	Alcohol-based gel	CDAD incidence	No change in CDAD rates	
Transmission Interruption – Disinfection						
Mayfield, 2000[46] USA	Before/after	3 hospital units; one unit with high incidence, 2 with lower	Hypochlorite solution for patient room cleaning	CDAD incidence	High incidence unit-CDAD decreased from 8.6/1,000 patient days to 3.3/1,000 patient days. No change in other units	No concurrent controls
Kaatz, 1988[47] USA	Outbreak	Hospital patients	Hypochlorite solution for patient room cleaning	CDAD incidence	Contamination decreased and the outbreak ended. Phosphate-buffered hypochlorite was effective for disinfection,	Before-after design in the setting of an epidemic
Struelens, 1991[48]	Before/after design	Hospital patients	Intensive cleaning measures, aldehydes	CDAD incidence	Protective efficacy 73% (CI 46% to 87%)	No concurrent controls
Multiple Interventions						
Abbett, 2009[49] USA	Prospective before-after study	Hospital patients	Infection control practices, laboratory	CDAD incidence	Use of a checklist of hospital interventions to	No concurrent control

Table 4. Prevention interventions (continued)

Author/Year Country	Study Design	Population	Intervention	Outcome	Findings	Quality Issues
			notification procedures, and steps coordinate infection control and environmental services aimed to decrease the transmission of *C. difficile* between patients (i.e., a prevention checklist		decrease the incidence of healthcare-associated CDAD	
Boyce, 2008[50] USA	Before/after time series	Hospital	Liquid vapor hydrogen peroxide decontamination system	CDAD incidence	Nosocomial CDAD incidence decreased and remained at lower levels	No concurrent control
Drudy, 2007[53] Ireland	Prospective time series (before/after)	Hospital patients	Antimicrobial use, enhanced surveillance, education, hand hygiene, equipment, intensified environmental cleaning	CDAD incidence	CDAD incidence decreased from a peak of 21 cases/1,000 patient admissions to 5/1,000 patient admissions	No concurrent controls
Valiquette, 2007[56] Canada	Retrospective time series (before/after)	Hospital patients	Antimicrobial use, education, isolation, equipment, intensified environmental cleaning	CDAD incidence	Nonrestrictive measures to optimize antibiotic usage (leading to decreases in usage) led to a decrease in CDAD incidence by 60%	Retrospective, no concurrent control
Whitaker, 2007[58] USA	Prospective time series (before/after)	Hospital patients	Antimicrobial use, education, isolation, automated report functions, and standardized nursing unit isolation processes	CDAD incidence	66% reduction in the number of health care–associated CDAD cases was achieved during the study	No concurrent controls
Zafar, 1998[57] USA	Prospective time series (before/after)	Hospital patients	Isolation, patient/staff movement, hand hygiene, patient room practices, intensified	CDAD incidence	Incidence of CDAD decreased by 60% from 1990 to 1996 following use of use of comprehensive infection control measures.	No concurrent controls

Table 4. Prevention interventions (continued)

Author/Year Country	Study Design	Population	Intervention	Outcome	Findings	Quality Issues
			environmental cleaning			
McNulty, 1997[54] UK	Retrospective time series (before/after)	Hospital patients, elderly care unit	Antimicrobial use, isolation, patient/staff movement, hand hygiene, patient room practices, intensified environmental cleaning	CDAD incidence	Thirty-seven cases of CDAD occurred in the period before and 16 in the period after policy change (combined approach of infection control and strict antibiotic policies).	Retrospective, no concurrent control
Cartmill, 1994[52] UK	Prospective time series (before/after)	Hospital patients	Antimicrobial use, enhanced surveillance, isolation, patient/staff movement, intensified environmental cleaning	CDAD incidence	Subsequent to the intervention measures, there was a substantial and sustained decreased in the incidence of CDAD	No concurrent controls
Pear, 1994[55] USA	Prospective time series (before/after)	Hospital patients (Veterans Affairs)	Antimicrobial use, education, isolation, patient room practices, intensified environmental cleaning	CDAD incidence	Nosocomial epidemic of CDAD was controlled by analysis of antibiotic use patterns and by subsequent restriction of clindamycin	No concurrent controls
Brown, 1990[51] USA	Retrospective time series (before/after)	Hospital patients	Antimicrobial policy, isolation	CDAD incidence	CDAD attack rate dropped progressively	Retrospective, no concurrent control

Table 5. (A) Studies of multiple interventions used together to reduce CDAD incidence

Study	Type of Study	Epidemic or Excessive Incidence?	CDAD incidence Decreased After intervention?	Interventions											
								Isolation (n=8)		Patient/Staff Movement (n=3)		Hand Hygiene (n=7)	
				Improved Antimicrobial Use	Enhanced Surveillance, Analysis, and Reporting of CDAD Data	Laboratory-based Infection Prevention Alert System	Infection Prevention Education	Enteric Precautions	Isolation	CDAD Patient Movement Restricted	Restriction of Nursing Cross-Cover	Enhanced Hand Washing	Alcohol-based Rub or Gel
Abbett, 2009[49]	Prospect time series (before/after)	Yes	Yes		X	X	X		X			X	
Boyce, 2008[50]	Prospect time series (before/after)	Yes	Yes	X	X			X	X			X	
Brown, 1990[51]	Retrospect time series (before/after	Yes	Yes	X				X					
Cartmill. 1994[52]	Prospect time series (before/after)	Yes	Yes	X	X				X	X			
Drudy, 2007[53]	Prospect time series (before/after)	Yes	Yes	X	X		X						X
McMullen, 2007[60]	Prospect time series (before/after)	Yes	Yes									X	
McNulty, 1997[54]	Retrospect time series (before/after	Yes	Yes	X				X	X		X	X	
Pear, 1994[55]	Prospect time series (before/after)	Yes	Yes	X			X						
Valiquette, 2007[56]	Retrospect time series (before/after	Yes	Yes	X			X		X				
Whitaker, 2007[58]	Prospect time series (before/after)	Yes	Yes	X	X		X		X			X	

Table 5. (A) Studies of multiple interventions used together to reduce CDAD incidence (continued)

Study	Type of Study	Epidemic or Excessive Incidence?	CDAD incidence Decreased After intervention?	Interventions									
								Isolation (n=8)		Patient/Staff Movement (n=3)		Hand Hygiene (n=7)	
				Improved Antimicrobial Use	Enhanced Surveillance, Analysis, and Reporting of CDAD Data	Laboratory-based Infection Prevention Alert System	Infection Prevention Education	Enteric Precautions	Isolation	CDAD Patient Movement Restricted	Restriction of Nursing Cross-Cover	Enhanced Hand Washing	Alcohol-based Rub or Gel
Zafar, 1998[57]	Prospect time series (before/after)	No	Yes						X	X		X	
Total number of studies evaluating specific intervention				**8**	**5**	**1**	**5**	**3**	**7**	**2**	**1**	**6**	**1**

Table 6. (B) Studies of multiple interventions used together to reduce CDAD incidence

Study	Type of Study	Interventions									
		Practices within Patient Rooms (n=5)			Equipment (n=4)		Intensified Environmental Cleaning (n=10)				
		Gloves for Patient Contact	Gowns or Aprons for Patient Contact	Visitors Comply with Infection Prevention Practices	Dedicated Equipment, Equipment Cleaning	Disposable Rectal Thermometers	Room Cleaning with Hypochlorite	Ward Cleaning with Hypochlorite	Room Cleaning with Hydrogen Peroxide	Ward Cleaning with Hydrogen Peroxide	Room Cleaning with Detergent	Unspecified
Abbett, 2009[49]	Prospect time series (before/after)		X	X	X		X					
Boyce, 2008[50]	Prospect time series (before/after)						X		X	X		
Brown, 1990[51]	Retrospect time series (before/after											
Cartmill, 1994[52]	Prospect time series (before/after)						X					
Drudy, 2007[53]	Prospect time series (before/after)				X		X					
McMullen, 2007[60]	Prospect time series (before/after)						X	X				
McNulty, 1997[54]	Retrospect time series (before/after	X	X								X	
Pear, 1994[55]	Prospect time series (before/after)	X										X
Valiquette, 2007[56]	Retrospect time Series (before/after				X	X	X		X	X		
Whitaker, 2007[58]	Prospect time series (before/after)			X	X		X	X				

Table 6. (B) Studies of multiple interventions used together to reduce CDAD incidence (continued)

Study	Type of Study	Interventions										
		Practices within Patient Rooms (n=5)			Equipment (n=4)		Intensified Environmental Cleaning (n=10)					
		Gloves for Patient Contact	Gowns or Aprons for Patient Contact	Visitors Comply with Infection Prevention Practices	Dedicated Equipment, Equipment Cleaning	Disposable Rectal Thermometers	Room Cleaning with Hypochlorite	Ward Cleaning with Hypochlorite	Room Cleaning with Hydrogen Peroxide	Ward Cleaning with Hydrogen Peroxide	Room Cleaning with Detergent	Unspecified
Zafar, 1998[57]	Prospect time series (before/after)	X	X	X								X
Total number of studies evaluating specific intervention		**3**	**3**	**3**	**4**	**1**	**7**	**2**	**2**	**2**	**1**	**2**

Table 7. Summary of risk factors for CDAD

Study	Specific Antibiotic Use	General Antibiotic Use	Health Status or Disease Severity	Acid Suppression	Hospitalization in an ICU	Age	Miscellaneous
Sundram, 2009[147]	*Ciprofloxacin OR = 3.72*						
Walbrown, 2008[142]	*Quinolones, pre vs. post change in formulary RR = 1.9, P* <0.001	*Pre vs. post change in formulary RR = 1.5, P* <0.001					
Munoz, 2007[150]							*Hypogamma-globulinemia RR = 5.8*
Peled, 2007[99]			*Functional capacity score OR = 9.1*	*PPI OR = 6.1 Histamine blocker OR = 3.1*			*Hypoalbuinemia OR = 3.8 Leukocytosis OR = 2.7*
Samore, 2006[146]	*Clindamycin OR = 4.2*						
Yearsley, 2006[148]		*OR = 13.1*	*OR = 1.90*				*Female gender OR = 1.79*
Vesta, 2005[143]		*No significant differences*	*Horn's Index P = 0.0022*				
Kyne, 2002[144]			*Severe underlying dz OR = 17.6*				
Mody, 2001[145]	*3rd generation cephalosporins OR = 3.6*						
Winston, 2001[151]	*Imipenem P = 0.02*						
Schwaber, 2000[149]	*Cephalosporin P = 0.03; 3rd generation cephalosporins P = 0.02*	*Greater number used P = 0.02*					
Katz, 1997[98]	*Cephalosporin P = 0.001*	*Antibiotic use past 30 days P =0.009; Antibiotic use prior to transfer/ admission P =0.009*		*P = 0.04*			

Table 7. Summary of risk factors for CDAD (continued)

Study	Specific Antibiotic Use	General Antibiotic Use	Health Status or Disease Severity	Acid Suppression	Hospitalization in an ICU	Age	Miscellaneous
Bignardi, 1998[14] Searched to March 1996		*Duration of antibiotic course; multiple antibiotics*	Severity of underlying diseases	Anti-ulcer medications	Yes	Yes	Non-surgical gastrointestinal procedures, nasogastric tube, hospital length of stay

Key Question 3. What are the comparative effectiveness and harms of different antibiotic treatments?

Search Results

Ten randomized clinical trials were identified that evaluated different antimicrobials (or different doses of a single drug) available for treatment of CDAD in the United States. These ten studies, published from 1978 to 2009, ranged in size from 39 to 172 subjects. Table 8 provides a breakdown of the trial comparators. Vancomycin is the most frequently studied antimicrobial, examined in eight of the ten studies. The most frequent comparison was vancomycin versus metronidazole (three studies, one of which also included fusidic acid and teicoplanin treatment arms, which are not included in this analysis), followed by two studies of vancomycin versus bacitracin. The remaining comparisons (vancomycin versus nitazoxanide, vancomycin high versus low dose, vancomycin versus placebo, metronidazole versus nitazoxanide, and metronidazole versus metronidazole plus rifampin,) all occurred in single studies. The typical study followup period was 21 to 31 days. The largest patient sample was 172, and most studies were in the range of approximately 40 to 60 patients. (See Appendix Table 4.)

Table 8. Summary of trial comparators for ten trials of antibiotic treatment of CDAD

	Vancomycin	Metronidazole	Nitazoxanide	Bacitracin	Metronidazole + Rifampin	Placebo
Vancomycin	1 (N=56) (dosing)					
Metronidazole	3 (N=172 N=62 N=101)	***				
Nitazoxanide	1 (N=50)	1 (N=142)	***			
Bacitracin	2 (N=62 N=42))			***		
Metronidazole + Rifampin		1 (N=39)			***	
Placebo	1 (N=44)					***

Key Points

- Overall, study quality is low.
- Vancomycin and metronidazole, the most frequently clinically used antimicrobials, were the most frequently compared antimicrobials.
- Three RCT comparisons of vancomycin to metronidazol, with a total of 335 pooled subjects, found no significant differences in any examined outcome.
- One RCT comparing vancomycin to metronidazole, using a pre-specified subgroup analysis of 69 patients, found a small but significant increase in the proportion of subjects with severe CDAD who achieved initial clinical cure with vancomycin.
- Harms were not reported with sufficient detail to compare the risks of any particular antimicrobial with another antimicrobial.
    - When harms were reported, they were generally nonserious (nausea, emesis, etc.) and transient.

Minor key points.

- No other head to head trial demonstrated superiority of any single antimicrobial for initial clinical cure, clinical recurrence, or mean days to resolution of diarrhea.
- Combination therapy with rifampin and metronidazole resulted in significantly higher mortality when compared to treatment with metronidazole only.
- Pooled data of 104 subjects comparing vancomycin to bacitracin showed significantly higher rates of organism or toxin clearance for vancomycin.
- No data were available to assess the importance of general patient characteristics or the strain of organism on the effectiveness of an antimicrobial.

Quality of the Studies

Overall study quality is low. Only one study specified that the investigators (who also assessed outcomes) were blinded with respect to treatment.[62] Quality summary tables are available in Appendix C of this report (see Appendix Tables 5 and 6). Strength of evidence is summarized in Appendix Tables 7 and 8.

Detailed Analysis

As vancomycin and metronidazole are the most frequently employed antimicrobials, and therefore of greatest interest to clinicians, results are broken into two sets: (1) vancomycin versus metronidazole and (2) all other comparisons of standard treatment trials.

Initial cure. The percentage of subjects initially cured with vancomycin ranged from 84 percent to 94percent among individual studies, with a mean value of 88 percent (Table 9). For subjects treated with metronidazole, the individual cure rates ranged from 73 percent to 94 percent, with a mean value of 81 percent. The relative risk for initial cure comparing vancomycin to metronidazole was 1.08 (95 percent CI 0.99 – 1.19).

Table 9. Initial clinical cure (# subjects / # randomized) for vancomycin vs. metronidazole

Study	Vancomycin	Metronidazole	RR [95% CI]
Zar, 2007[62]	69/82 (84)	66/90 (73)	1.15 [0.98 to 1.34]
severe disease	*30/31 (97)*	*29/38 (76)*	*1.27 [1.05 to 1.53]*
Wenisch, 1996[65]	29/31 (94)	29/31 (94)	1.00 [0.88 to 1.14]
Teasley, 1983[68]	51/56 (91)	39/45 (87)	1.05 [0.91 to 1.21]
Totals	149/169 (88)	134/166 (81)	1.08 [0.99 to 1.19]

With the exception of vancomycin versus placebo, no other treatment comparison resulted in significant differences in initial clinical cure (Table 10).

Table 10. Initial clinical cure (# subjects / # randomized) for all other standard treatment trials

Study	Treatment 1	Treatment 2/ Control	RR (95% CI)
1. Vancomycin vs. nitazoxanide			
Musher, 2009[61]	20/27 (74)	17/23 (74)	1.00 [0.72 to 1.39]
severe disease	*7/10 (70)*	*8/10 (80)*	*0.88 [0.53 to 1.46]*
2. Vancomycin vs. bacitracin			
Dudley, 1986[66]*	15/23 (65)	12/16 (75)	0.87 [0.58 to 1.31]
Young, 1985[69]	18/21 (86)	16/21 (76)	1.13 [0.84 to 1.51]
Totals	33/44 (75)	28/37 (76)	1.01 [0.79 to 1.28]
3. Vancomycin high-dose vs. vancomycin low-dose			
Fekety, 1989[67]	22/28 (79)	24/28 (86)	0.92 [0.72 to 1.17]
4. Vancomycin vs. placebo			
Keighley, 1978[70]*	9/12 (75)	1/9 (11)	6.75 [1.03 to 44.08]
5. Metronidazole vs. nitazoxanide			
Musher, 2006[64]	28/44 (64)	68/98 (69) *36/49 (73) 7-day* *32/49 (65) 10-day*	0.92 [0.71 to 1.19]
6. Metronidazole vs. metronidazole plus rifampin			
Lagrotteria, 2006[63]	13/20 (65)	12/19 (63)	1.03 [0.64 to 1.65]

Clinical recurrence. The percentage of subjects meeting the investigator-determined definition of recurrent disease (after meeting criteria for initial cure) ranged from 7 percent to 17 percent with vancomycin, with a mean value of 11 percent. For metronidazole the range was from 5 percent to 21 percent, with a mean value of 12 percent. (Table 11) The relative risk for recurrence after vancomycin treatment compared to metronidazole was 0.92 (95 percent CI 0.47 – 1.77)

Table 11. Clinical recurrence: # subjects / # initially cured (percent) for vancomycin vs. metronidazole

Study	Vancomycin	Metronidazole	RR [95% CI]
Zar, 2007[62]	5/69 (7)	9/66 (14)	2.29 [0.49 to 10.76]
severe disease	*3/30 (10)*	*6/29 (21)*	*0.48 [0.13 to 1.75]*
Wenisch, 1996[65]	5/29 (17)	5/29 (17)	1.00 [0.32 to 3.09]
Teasley, 1983[68]	6/51 (12)	2/39 (5)	0.53 [0.19 to 1.50]
Totals	16/149 (11)	16/134 (12)	0.92 [0.47 to 1.77]

No other treatment comparison resulted in significant differences in clinical recurrence. (Table12)

Table 12. Clinical recurrence: # subjects / # initially cured (percent) for all other standard treatment trials

Study	Treatment 1	Treatment 2/ Control	RR (95percentCI)
1. Vancomycin vs. nitazoxanide			
Musher, 2009[61]	2/20 (10)	1/17 (6)	1.70 [0.17 to 17.16]
severe disease	*1/10 (10)*	*1/10 (10)*	*1.00 [0.07 to 13.87]*
2. Vancomycin vs. bacitracin			
Dudley, 1986[66]*	3/15 (20)	5/12 (42)	0.48 [0.14 to 1.62]
Young, 1985[69]	6/18 (33)	5/12 (42)	0.80 [0.31 to 2.04]
Totals	9/33 (27)	10/24 (42)	0.65 [0.31 to 1.35]
3. Vancomycin high-dose vs. vancomycin low-dose			
Fekety, 1989[67]	4/22 (18)	5/24 (21)	0.87 [0.27 to 2.84]
4. Vancomycin vs. placebo			
Keighley, 1978[70]*	NR	NR	
5. Metronidazole vs. nitazoxanide			
Musher, 2006[64]	8/28 (29)	14/68 (21) *9/36 7-day* *5/32 (3) 10-day*	1.39 [0.66 to 2.93]
6. Metronidazole vs. metronidazole plus rifampin			
Lagrotteria, 2006[63]	5/13 (38)	5/12 (42)	0.92 [0.35 to 2.41]

Mean days to resolution of diarrhea. Two of the three vancomycin versus metronidazole studies reported the mean time to resolution of diarrhea.[65,68] No differences were seen between treatment arms. (Table 13)

Table 13. Mean days to resolution of diarrhea/clinical improvement for vancomycin vs. metronidazole

Study	Vancomycin	Metronidazole	WMD [95% CI]
Zar, 2007[62]	Not reported	Not reported	
Wenisch, 1996[65]	3.1 ± 1.1	3.2 ± 1.1	0.10 [-0.65 to 0.45]
Teasley, 1983[68]	2.8 ± 1.8	2.4 ± 1.9	-0.40 [-0.35 to 1.15]
Totals			0.07 [-0.37 to 0.52]

* Subjects without demonstrable *C. difficile* cytotoxin and/or positive culture for *C. difficile* were removed and not included in the efficacy analyses.

No other treatment comparison resulted in significant differences in mean days to resolution of diarrhea. (Table 14)

Table 14. Mean days to resolution of diarrhea/clinical improvement for all other standard treatment trials

Study	Treatment 1	Treatment 2/ Control	WMD (95percentCI)
1. Vancomycin vs. nitazoxanide			
Musher, 2009[61]	Not reported	Not reported	
2. Vancomycin vs. bacitracin			
Dudley, 1986[66]	NR	NR	
Young, 1985[69]	4.3 ± 1.8	4.8 ± 1.8	-0.50 [-1.59 to 0.59]
Totals			-
3. Vancomycin high-dose vs. vancomycin low-dose			
Fekety, 1989[67]	4.3 ± 1.8	3.8 ± 1.4	0.50 [-0.44 to 1.44]
4. Vancomycin vs. placebo			
Keighley, 1978[70]	NR	NR	
5. Metronidazole vs. nitazoxanide			
Musher, 2006[64]	NR	NR	
6. Metronidazole vs. metronidazole plus rifampin			
Lagrotteria, 2006[63]	6.6	7.0	p = 0.73

All cause mortality. Mortality was rare overall, in part due to the short study followup periods. There were five deaths in each arm among the 335 subjects enrolled in studies comparing vancomycin to metronidazole (Table 15). Wenisch[65] evaluated four drugs including two not evaluated in this review but did not provide mortality data by subject. Depending on in which study arm the mortalities occurred in the Wenisch study,[65] there were between 10 and 13 total deaths in studies comparing vancomycin to metronidazole. Even if all three deaths in this study occurred in one arm, the difference in mortality could not reach statistical significance.

Table 15. All cause mortality (# subjects / # randomized) for vancomycin vs. metronidazole

Study	Vancomycin	Metronidazole	Nitazoxanide	Bacitracin	Placebo
Zar, 2007[62]	3/82 (4)	5/90 (6)			
Wenisch, 1996[65]	3 subjects died within first days of therapy (treatment groups not noted)				
Teasley, 1983[68]	2/56 (4)	0/45			

All cause mortality was significantly higher for combination metronidazole plus rifampin versus metronidazole alone (32 percent versus 5 percent).[63] There were no differences in all cause mortality in any of the other treatment comparisons (Table 16).

Table 16. All cause mortality (# subjects / # randomized) for all other standard treatment trials

Study	Vancomycin	Metronidazole	Nitazoxanide	Bacitracin	Placebo
Musher, 2009[61]	Overall mortality was 4percent (2/49 subjects) (treatment groups not noted)				
Lagrotteria, 2006[63]		1/20 (5) 6/19 (32) + Rif			
Musher, 2006[64]	1+/44†		3+/98†		
Fekety, 1989[67]	1/28 HD 1/28 LD				
Dudley, 1986[66]	0/31	1/31 (3)			
Young, 1985[69]	0/21	0/21			
Keighley, 1978[70]	0 “colitis”/12	0 “colitis”/12			

†A total of 13 (9percent) deaths occurred but only the 4 deaths above were denoted by treatment arm

Other outcomes. Where the outcomes were reported, no differences were found between vancomycin and metronidazole for clearance of toxin,[65] laboratory-confirmed relapse,[65] or persistence of the organism[68] (Table 17).

Table 17. Other outcomes (# subjects / # assessed) for vancomycin vs. metronidazole

Study	Vancomycin	Metronidazole	RR [95percentCI]
Zar, 2007[62]	Not reported	Not reported	
Wenisch, 1996[65]	CT at day 6 22/31 (71)	CT at day 6 22/31 (71)	1.00 [0.73 to 1.37]
	LR at day 30 9/31 (29)	LR at day 30 9/31 (29)	1.00 [0.46 to 2.18]
Teasley, 1983[68]	P at day 21 11/43 (26)	P at day 21 14/35 (40)	0.64 [0.33 to 1.23]
Totals			-

CT: clearance of toxin. LR: laboratory-confirmed-relapse. P: persistence.

Pooled data of 104 subjects comparing vancomycin to bacitracin showed significantly higher rates of organism or toxin clearance for vancomycin.[66,69] No other differences were found in reported outcomes (Table 18).

Harms. Reported adverse events were relatively uncommon, minor, and not associated with one drug compared with the other. One study reported two episodes of intolerance (nausea and vomiting) leading to subject withdrawal, one in each treatment arm.[68] Another reported a subject with emesis which developed while on metronidazole, which resolved when treatment was changed to vancomycin; in the same study another subject developed nausea while on vancomycin, which resolved when treatment was changed to metronidazole.[62] The third study reported “gastrointestinal discomfort” (which did not result in cessation of therapy) in 10 percent of subjects receiving metronidazole, compared to none with vancomycin, a difference which did not reach significance.

Disease severity. Only one study stratified patients by disease severity at the time of screening.[62] Severity was dichotomized into two outcomes: mild or severe disease. This trial stratified treatment based on disease severity (mild versus severe). Sixty-nine subjects, 31 who received vancomycin and 38 who received metronidazole, met the pre-specified definition of severe disease. Patients with two or more of the following were considered to be severe: 60 years

of age or over, temperature over 38.3 degrees Celsius, albumin level less than 2.5 mg/dL, or peripheral white blood count greater than 15,000 cells/mm^3 within 48 hours. Initial cure was more common among those receiving vancomycin (97 percent versus 76 percent), with a relative risk for initial cure of 1.27 (95 percent CI, 1.05 to 1.53) (Table 9). No other significant differences in outcomes were found by disease severity.

C. difficile strain. Our search did not identify any evidence assessing the comparative effectiveness of different treatments stratified by disease strain.

Patient characteristics. Our search did not identify any evidence for comparative effectiveness by general patient characteristics such as age, gender, or treatment setting.

Resistance of other pathogens. The impact of treatment for CDAD on other pathogens has not been addressed by the available studies that directly assigned subjects to different drugs. From observational studies, there is some evidence that treatment with either metronidazole or vancomycin can cause an increase in the incidence in the carriage of vancomycin resistant enterococci;[158,159] however, the magnitude of this effect and the clinical significance is uncertain.

Table18. Outcomes for all standard treatment trials

Study	Vancomycin	Metronidazole	Nitazoxanide	Fusidic Acid	Placebo
Clinical initial cure (# subjects / # randomized)					
Musher, 2009[61]	20/27 (74)		17/23 (74)		
Zar, 2007[62]	69/82 (84)	66/90 (73)			
Lagrotteria, 2006[63]		13/20 (65) 12/19 (63) + Rif			
Musher, 2006[64]		28/44 (64)	68/98 (69)		
Wullt, 2004[160]		51/64 (80)		49/67 (73)	
Wenisch, 1996[65]	29/31 (94)	29/31 (94)		27/29 (93)	
de Lalla, 1992[161]	20/24 (83)				
Fekety, 1989[67]	22/28 (79) HD 24/28 (86) LD				
Dudley, 1986[66]	15/23* (65)				
Young, 1985[69]	18/21 (86)				
Teasley, 1983[68]	51/56 (91)	39/45 (87)			
Keighley, 1978[70]*	9/12* (75)				1/9* (11)
Clinical recurrence (# subjects / # initially cured) for all standard treatment trials					
Musher, 2009[61]	2/20 (10)		1/17 (6)		
Zar, 2007[62]	5/69 (7)	9/66 (14)			
Lagrotteria, 2006[63]		5/13 (38) 5/12 (42) + Rif			
Musher, 2006[64]		8/28 (29)	14/68 (21)		
Wullt, 2004[160]		15/51 (29)		14/49 (29)	
Wenisch, 1996[67]	5/29 (16)	5/29 (16)		8/27 (30)	
de Lalla, 1992[161]	4/20 (20)				
Fekety, 1989[67]	4/22 (18) HD 5/24 (21) LD				
Dudley, 1986[66]	3/15 (20)				
Young, 1985[69]	6/18 (33)				
Teasley, 1983[68]	6/51 (12)	2/39 (5)			
Keighley, 1978[70]	Not reported	Not reported			
All-cause mortality (# subjects / # randomized) for all standard treatment trials					
Musher, 2009[61]	Overall mortality was 4% (2/49 subjects) (treatment groups not noted)				
Zar, 2007[62]	3/82 (4)	5/90 (6)			
Lagrotteria, 2006[63]		1/20 (5) 6/19 (32) + Rif			
Musher, 2006[64]	1+/44†		3+/98†		
Wullt, 2004[160]		0/64		0/67	
Wenisch, 1996[65]	3 subjects died within first days of therapy (treatment groups not noted)				
de Lalla, 1992[161]	2 subjects died within first days of therapy (treatment groups not noted)				
Fekety, 1989[67]	1/28 HD 1/28 LD				

Table18. Outcomes for all standard treatment trials (continued)

Study	Vancomycin	Metronidazole	Nitazoxanide	Fusidic Acid	Placebo
Dudley, 1986[66]	0/31	1/31 (3)			
Young, 1985[69]	0/21	0/21			
Teasley, 1983[68]	2/56 (4)	0/45			
Keighley, 1978[70]	0 “colitis”/12	0 “colitis”/12			
Mean days to resolution of diarrhea/clinical improvement					
Musher, 2009[61]	NR		Not reported		
Zar, 2007[62]	NR	NR			
Lagrotteria, 2006[63]		6.6 7.0 + Rif			
Musher, 2006[64]		Not reported	Not reported		
Wullt, 2004[160]		Not reported		Not reported	
Wenisch, 1996[65]	3.1 ± 1.1	3.2 ± 1.1		3.8 ± 1.6	
de Lalla, 1992[161]	3.6 ± 1.7				
Fekety, 1989[67]	4.3 ± 1.8 HD 3.8 ± 1.4 LD				
Dudley, 1986[66]	Not reported				
Young, 1985	4.3 ± 1.8				
Teasley, 1983[68]	2.8 ± 1.8	2.4 ± 1.9			
Keighley, 1978[70]	Not reported				Not reported
Clearance of organism (CO) / toxin (CT) or laboratory-confirmed-relapse (LR) / persistence (P) for evaluable subjects					
Musher, 2009[61]	Not reported		Not reported		
Zar, 2007[62]	Not reported	Not reported			
Lagrotteria, 2006[63]		LR 2 LR 4 (+ Rif)			
Musher, 2006[64]		Not reported	Not reported		
Wullt, 2004[160]		CT 41/53 (77) P 8/50 (16)		CT 46/59 (78) P 4/46 (9)	
Wenisch, 1996[65]	CT 22/31 (71) LR 9/31 (29)	CT 22/31 (71) LR 9/31 (29)		CT 14/29 (48) LR 15/29 (52)	
de Lalla, 1992[161]	P 9/20 (45)				
Fekety, 1989[67]	O 4/10 (40) HD O 5/9 (56) LD				
Dudley, 1986[66]	O 11/14 (79) T 12/14 (86)				
Young, 1985[69]	O 17/21 (81) T 15/18 (83)				
Teasley, 1983[68]	P 11/43 (26)	P 14/35 (40)			
Keighley, 1978[70]	O 11/12 (92) T 12/12 (100)				O 1/9 (11) T 3/9 (33)

HD = high dose; LD = low dose; Rif = Rifampin. * Subjects without demonstrable *C. difficile* cytotoxin and/or positive culture for *C. difficile* were removed and not included in the efficacy analyses. **4 subjects excluded with no reasons given. †A total of 13 (9%) deaths occurred but only the 4 deaths above were denoted by treatment arm

Key Question 4. What are the effectiveness and harms of nonantibiotic adjunctive interventions?

Search Results

A total of six RCTs on nonantibiotic adjunctive treatments of CDAD (Table 19) and 12 studies addressed prevention of CDAD (Table 20) formed the basis of this analysis. Five of the studies on treatment of CDAD compared a nonantibiotic intervention with an active control, i.e., a standard antibiotic treatment for CDAD, oral vancomycin or metronidazole.[71-75] One study compared a nonantibiotic intervention with placebo.[76] All but one[73]of the 12 prevention studies compared the nonantibiotic intervention to placebo rather than to another intervention, reflecting the current state of this area of science. Numerous published case reports, as well as nonexperimental studies, describe additional nonantibiotic approaches for treatment of CDAD and their possible harms. (Table 21)

Due to the heterogeneity of the interventions, quantitative analysis was not possible. We therefore provide a narrative review of the literature.

Key Points

- Overall, study quality was low.
- *C. difficile* immune whey, and colestipol, are not more effective in treating CDAD than standard antibiotic treatment with oral vancomycin or metronidazole or placebo.
- Administration of a probiotic to treat CDAD in critically ill patients increases risk for greater morbidity and mortality from fungemia without any known benefit.
- There is low quality limited evidence that the nonantibiotic interventions in this review are not more effective than placebo for primary prevention of CDAD.
- There is low quality limited evidence from one subgroup analysis that a prebiotic may reduce diarrhea recurrence in patients treated for CDAD more so than placebo with standard antibiotics.
- There is limited moderate quality evidence from one study that monoclonal antibodies are effective in preventing recurrence of CDAD.
- There is limited low quality evidence from two case series that fecal biomass transplant is effective in treating recurrent CDAD for up to 1 year.
- Definitions of CDAD with regard to diarrhea, i.e., number and consistency of stool, were inconsistent across studies.

Quality of the Studies

The level of the quality of the evidence is low. Several study limitations lowered the quality of their findings. Among the most common were lack of a power analysis, inadequate power to detect significant differences, lack of an intent to treat analysis, and failure to define allocation concealment. In one study, the findings of subjects with CDAD at the start of a nonantibiotic intervention were combined with those who developed CDAD after the intervention.[76] The problem of a nonstandardized, incomplete, or unspecified definition of CDAD has already been noted. In one study, a culture of *C. difficile* (which could have indicated a nontoxigenic strain of

the organism) was accepted in place of, or in addition to, a toxin test for the definition of CDAD for some patients.[76] Longer term followup for CDAD incidence or recurrence sometimes relies on reports of diarrhea without re-testing for *C. difficile* toxin. There was lack of standardization of the active control in two studies, allowing subjects to receive an antibiotic for CDAD as prescribed by their physicians.[83,85] Summaries of study quality and strength of evidence are provided in Appendix Tables C9 and C10.

Detailed Analysis

Defining the outcome of CDAD. The operative definition of diarrhea, which is part of the definition of CDAD, varied among the studies for prevention and treatment of CDAD (Tables 19 and 20). Seven (39 percent) of the studies defined diarrhea as three or more loose or liquid stools per day for 2 days.[71-73,75-77,85] One study required that same number and consistency of stools but for only one day,[78] and another study did not require the three stools per day to be loose or liquid.[75] One study required two liquid stools on 3 or more days.[82] The most liberal definition of diarrhea was one to two loose stools per day.[83] Diarrhea due to *C. difficile* was not explicitly defined in four (6 percent) studies.[74,79-81]

Treatment of CDAD. The effectiveness of three types of nonantibiotic interventions were compared for treating CDAD: (1) agents that absorb or bind *C. difficile* toxins,[73,76] (2) an immune whey to enhance the immune response to *C. difficile* toxins,[74] and (3) probiotics that aim to recolonize the intestinal flora with nonpathogenic bacteria[71,72,75] (Table 19). All interventions were administered orally. Probiotics were the only intervention administered as an adjunct to standard antibiotic treatment for CDAD;[71,72,75] the two other nonantibiotic interventions were administered independently. The probiotic in two studies contained *Sacchromyces boulardii*[71,75] and in one it contained *Lactobacillus plantarum.*[72]

Subjects in the treatment studies had a mean age ranging from 58 to 67 years. Females comprised more than 70 percent of the sample in three of the six studies,[71,72,75] and, in one study, the age and gender of subjects were not reported.[76] Subjects were hospital in-patients in three studies.[71,75,76]

The findings of the studies in Table 19 are presented in the same direction, i.e., as CDAD resolution (versus treatment failure) to facilitate comparison and interpretation. In five of the six studies of CDAD treatment, the main outcome was the incidence of resolving CDAD, which was defined as diarrhea in patients with a positive stool test for *C. difficile* toxin; in one study, time to resolution of CDAD was the primary outcome.[73]

Treatment of primary CDAD. In the comparative treatment studies, CDAD resolved in 50 to100 percent of cases across interventions. The rate of resolution of CDAD was the lowest in the study, comparing an absorptive resin (25 percent of subjects) to placebo (21 percent of subjects).[76] No statistical testing results were reported by three of the treatment studies.[72,74,76] There was no significant difference in the resolution of CDAD between the interventions compared in any of the studies[71-74] based on reported statistics or those conducted by the reviewers.

Treatment of recurrent CDAD. In two comparative treatment studies the subjects recruited were treated for a recurrent (rather than an initial) episode of CDAD.[71,72] A third study conducted a subanalysis of their subjects with recurrent CDAD.[75] In all three studies, the nonantibiotic intervention was probiotic. There was no significant difference in CDAD between subjects who were administered a probiotic and a standard antibiotic for CDAD compared to those who

received an antibiotic alone.[71,72] In the study that analyzed a subset of their patients with recurrent CDAD, a significantly higher percentage of subjects on a standard antibiotic plus a probiotic resolved diarrhea compared to those on a standard antibiotic and a placebo.[75]

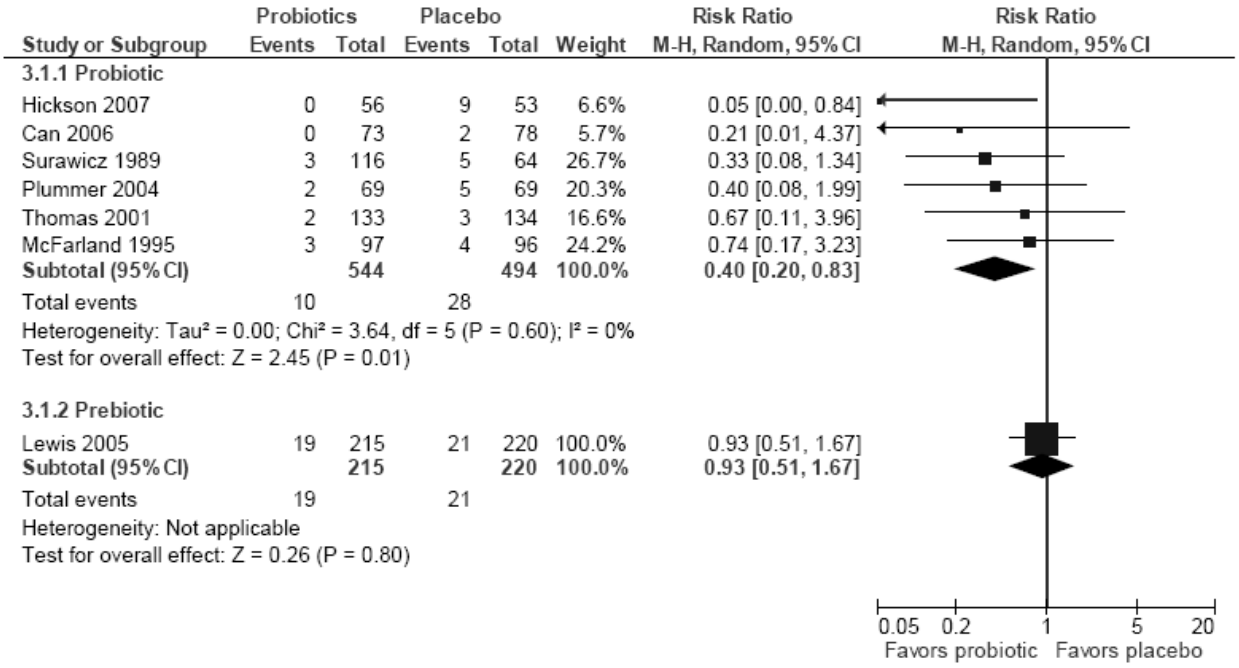
Prevention of primary CDAD. The nonantibiotic interventions investigated for preventing CDAD were (1) probiotics,[77-82] (2) a prebiotic (oligofructose) that aims to support a normal ecology of bacteria,[83,84] (3) a *C. difficile* cytotoxin binder (Tolevamer),[73] and (4) a monoclonal antibody to *C. difficile* toxins[85] (Table 20). Six different probiotics were tested, and in two of the eight studies, the probiotic contained more than one strain of bacteria.[80,82] Seven of the 12 CDAD prevention trials using nonantibiotic interventions focused on primary prevention, i.e., avoiding a first occurrence of CDAD.[77-83] All of the studies of primary prevention of CDAD investigated either a probiotic (six studies) or a prebiotic (one probiotic). Three studies that tested a nonantibiotic intervention for treating CDAD also investigated its ability to prevent CDAD recurrence.[71-73]

Subjects in the primary prevention studies had a mean age of 47 to 77 years. Females comprised less than one-third of the sample in two studies[77,81]and, in one study, the age and sex of the sample were not reported.[80] Subjects in all of the primary prevention studies were hospitalized patients.

The overall incidence of CDAD across intervention groups was relatively low, ranging from 2 percent to 9 percent. Only one of seven studies, which investigated a mixture of two probiotics (*L. casei and S. thermophilus*), showed a significantly lower incidence of CDAD diarrhea compared to placebo;[82] the investigators of this study acknowledged that the study was underpowered to detect a significant difference greater than by chance. In four studies, statistical testing was not reported.[78,79,81,83] We did not pool the studies due to the heterogeneity of the interventions. Based on reported statistics or those conducted by the reviewers, there was no significant difference in the recurrence of CDAD between any of the interventions and placebo in the six other studies.[77-81,83]

There is disagreement in the research community regarding the appropriateness of pooling results of probiotics due to the heterogeneity of probiotic organisms used and variability in dosing. We provide a forest plot (Figure 5) of the effects of probiotics on overall incidence of CDAD from the primary prevention probiotic trials for those who view such aggregation as reasonable. The pooled RR is 0.40 (95 percent CI, 0.20, 0.83). The prebiotic trial showed no effect.

Figure 5. Overall incidence of CDAD from probiotic primary prevention trials



Prevention of recurrence of CDAD. Five studies investigated the effectiveness of a nonantibiotic intervention to prevent the recurrence of CDAD (Table 20). Two studies investigated a probiotic,[71,72] one a prebiotic,[84] one a toxin binder,[73] and one a monoclonal antibody to *C. difficile*.[85] The mean age of subjects ranged from 63 to 75 years. Females comprised 70 percent or more of the subjects in two studies.[71,72] Hospital inpatients comprised the sample in one study[83] and were included along with nonhospitalized subjects in a second study.[71] The overall recurrence rate of CDAD across intervention groups ranged from 6.5 percent to 34.5 percent.

A significantly lower rate of CDAD recurrence was reported in two studies following administration of the prebiotic oligofructose[84] or a monoclonal antibody to *C. difficile* toxins A and B.[85] In both studies, the recurrence rate of CDAD was approximately three times greater in subjects on placebo compared to the intervention. There was no significant difference in the recurrence of CDAD in subjects taking probiotics[71,72] or a *C. difficile* cytotoxin binder[73] compared to controls.

Additional nonantibiotic approaches. In addition to the nonantibiotic interventions for CDAD addressed in this review, case reports, or nonexperimental studies reveal numerous other approaches for treating or preventing CDAD (Table 21). Use of other probiotics (for example, yogurt containing live bacterial cultures)[162,163] and other cytotoxin absorbing resins[164] have been reported.

Another approach that is currently under investigation is fecal biomass transplant which instills feces from a healthy donor into the colon of a patient with CDAD.[86,87,165] In two case series with a total of 24 patients; 20 patients resolved diarrhea and 18 of 22 patients with stool samples were negative for *C. difficile* toxin.

Other nonantibiotic interventions include a monoclonal antibody to *C. difficile* toxin A,[166] intravenous immunoglobulin G,[167,168] a macrocycle (bacteriocidal fermentation) agent,[169] a *C. difficile* toxoid vaccine,[170] and a nontoxigenic strain of *C. difficile*.[171]

Potential harms. Harmful effects of nonantibiotic interventions for CDAD appear to be few, but not all studies or case reports included adverse effects in their finding (Tables 19-21). A serious potential harm associated with administration of probiotics for CDAD in critically ill patients is fungemia.[172,173] In one review of an outbreak, previous medical charts, and the literature, 46 percent of 60 critically ill patients who developed fungemia had been administered a probiotic, and 28 percent subsequently died.[172] In addition, McFarland reported finding 12 cases of *Lactobacillus* bacteremia in patients (mostly children) taking a probiotic containing *Lactobacillus*.[10] Minor adverse symptoms of probiotics and prebiotics were abdominal symptoms such as nausea, bloating, and vomiting, and they have not differed significantly from those of subjects receiving placebo or an active control.[10,75,83] Headache (one subject), and abdominal pain, change in bowel habit, and polymyalgia rheumatica (one subject) occurred following *C. difficile* vaccination.[170] Hypotension, diarrhea, headache, nausea, and abdominal discomfort were reported after administration of a monoclonal antibody to *C. difficile* toxin A.[166]

Table 19. Nonantibiotic intervention for treatment of *C. difficile*

Study	Sample	Intervention/ Comparison and Method	Resolution of CDAD* (Diarrhea and CD toxin Positive Stool)	Other outcomes	Study Quality
Non-antibiotic intervention versus active control					
Louie, 2006[73] Clinical Infectious Diseases Multinational (United States, Canada, United Kingdom) Phase II, double-dummy	289 adults with diarrhea (≥3 loose/watery stools/d in the day preceding enrollment) and CD toxin positive or pseudo-membranes by colonoscopy and stool positive for *C. difficile* toxin 7 days before or at study entry (allowed 7 days for positive result to be found) 222 completed the study (77%) Age (median): 67 years Gender: Male 45% Race: White 91%	*C. difficile* toxin binder Tolvemar 3 g, high–molecular weight, anionic polymer (1400 kDa) that noncovalently binds *C. difficile* toxins A and B (1 g three times per day) (n=94) Tolvemar 6 g (2 g three times per day) (n=91) Vancomycin 500 mg (125 mg 4x/d x 10 d and matching placebo (n=94) All for no more than 28 days (d) Stool assessed daily Resolution of CDAD (i.e., efficacy of Tolvemar) defined as 2 consecutive days of hard or formed stools or ≤2 loose/watery stools/d Time to resolution of CDAD in number of days	Per-protocol analysis group Tolvemar 3g: 67% (48/72) subjects Tolvemar 6g: 83% (58/70) (p=.02 vs. 3g tolvemar) Vancomycin: 91% (73/80) (p=0.01 vs. 3g tolvemar, p=0.18 vs. 6 g tolvemar)	Time to resolution of CDAD: <u>Full analysis</u>; Tolvemar 6 g was inferior to vancomycin (p=0.53) and tolvemar 3 g was similar to the other treatments (p=0.09) <u>Per completed protocol</u>; Tolvemar 6 g was not inferior to vancomycin (p=0.03) Tolvemar 3g was inferior to Tolvemar 6 g (p=0.02) and vancomycin (p<0.01)	Allocation concealment: Not defined Blinding: double Intention-to-treat analysis: no, those with severe CDAD were excluded Withdrawals and dropouts adequately described: yes Notes: Different definitions of diarrhea for efficacy of intervention and recurrence of CDAD
Mattila, 2008[74] Scand J Infect Dis FInland	40 adults with ≥2 episodes of CDAD in past 3 months. and stool positive for *C. difficile* toxin 38 completed the study (95%) Mean age: 61.3 (CDIW 56.4 vs. metronidazole 65.7)	*C. difficile* immune whey (CDIW) CDIW 200 ml liquid and placebo tablets three times per day x 14 d (n=18) Metronidazole 400 mg	Response to study drugs at day 14 CDIW: 89% (16/18) Metronidazole: 100% (20/20) No statistical testing	Response to study drugs at day 28 (14 days after treatment) CDWI: 61% (11/18) Metronidazole:10% (2/20)	Allocation concealment: not defined Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes

Table 19. Nonantibiotic intervention for treatment of *C. difficile* (continued)

Study	Sample	Intervention/ Comparison and Method	Resolution of CDAD* (Diarrhea and CD toxin Positive Stool)	Other outcomes	Study Quality
	Gender: Male 47%	tablets and placebo liquid three times per day x 14 days (n=20) CD culture and toxin on days 0, 14, and 28 followup x 7 days Daily stool and symptom diary daily for 42 days; Followup after day 28 used stool and symptom diary only	reported, Fisher's Exact test performed by reviewers p=0.22		Notes: Sample size not achieved because of bankruptcy of sponsor Although CD culture and toxin were measured and stool diary data collected at day 14, the reported primary endpoint, "response to …study drugs" was not defined nor defined in relation to these measures; the same is true for sustained response at day 28 Secondary outcome of time to treatment failure through day 70 was measured by diarrhea only and not by CD toxin in stool CDIW had no local or systemic side effects
Adjuvant non-antibiotic intervention versus active control					
Surawicz, 2000[71] Clin Infectious Diseases United States	168 randomized adult inpatients and outpatients, 32 with recurrent CDAD (This paper reported subgroup analysis of treatment of subjects with recurrent CDAD only) *Recurrent CDAD subjects* Mean age (years): 61.6 Gender: Male (M) 41%	Probiotic-*Sacchromyces boulardii* (1 g/d) + high dose oral vanomycin (2g/d) (n=16) Placebo (1 g/d) + high dose oral vanomycin (2g/d) (n=16) Probiotic/placebo started on day 7 - day 28 CDAD was defined as	Probiotic and vancomycin: 13/16 (81.3%) Placebo and vancomycin: 8/16 (50%) Fisher's Exact test performed by reviewers p =0.06		Allocation concealment: adequate, centralized Blinding: double Intention-to-treat analysis: yes Withdrawals and dropouts adequately described: none reported

Table 19. Nonantibiotic intervention for treatment of *C. difficile* (continued)

Study	Sample	Intervention/ Comparison and Method	Resolution of CDAD* (Diarrhea and CD toxin Positive Stool)	Other outcomes	Study Quality
	All subjects Mean age: 63.2 Gender: Male 29%	diarrhea (≥3 loose/watery stools/s x 2 days or >8 loose stools/d within 48 hours) and positive CD assay (culture then toxin A or B) measured at multiple time points			
Wullt, 2003[72] Scan J Infect Dis Sweden	29 adult patients with recurrent disease from 9 centers (positive CD toxin assay within 6 days of enrollment at least 1 prior episode CD diarrhea within past 2 months and ongoing diarrhea). 8 patients (28%) lost to followup were not included in analysis, 21 completed trial (72%) Mean age: 63.8 Gender: Male 5%	Probiotic-*Lactobacillus plantarum* in fruit drink with oats fermented by L. plantarum 299v (5 x 10^{10} cfu) x 38 days and Metronidazole (400 mg three times per day po) x 10 d Placebo fruit drink with chemically acidified oats and metronidazole Toxin testing on days 11-13 followup about diarrhea on days 37-41 and 70-75 Clinical cure was defined as no diarrhea (≥3 loose stools x 2 days) on days 5-10 of treatment:	Probiotic and metronidazole: 92% (11/12) Placebo and metronidazole: 100% (9/9) No statistical testing reported, Fisher's Exact test performed by reviewers p=1.0		Allocation concealment: Not defined Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes Notes: CD toxin not measured after day 11
McFarland, 1994[75] JAMA United States	124 in patients with active CDAD and receiving standard antibiotic treatment (vancomycin or metronidazole) 104 (84%) completed the study Mean age 58.1 Gender: Male 23% 64 patients had initial CDAD and	Probiotic-Lyophilized *S. boulardii* 3x10^{10} cfu (1 g) orally in two 250 mg capsules/days x 4 weeks and standard therapy, vancomycin or metronidazole or both (n=57). Probiotic was given within 4 days of treatment	Resolution of CDAD *Overall* (all subjects, n=124) Probiotic: 73.4% (42/57) Placebo: 55.2% (37/67) p = 0.05 *Subgroup analysis* Subjects treated for recurrent CD (n=60)		Allocation concealment: Adequate, blinded study drug kits Blinding: double Intention-to-treat analysis: yes Withdrawals and dropouts adequately described: yes Notes: No difference in

Table 19. Nonantibiotic intervention for treatment of *C. difficile* (continued)

Study	Sample	Intervention/ Comparison and Method	Resolution of CDAD* (Diarrhea and CD toxin Positive Stool)	Other outcomes	Study Quality
	60 had recurrent CDAD	Placebo and standard therapy, vancomycin or metronidazole or both (n=67). Both groups followed for 4 weeks and an additional 4 weeks CDAD defined as diarrhea ≥3 stools/d x 2 consecutive days and 1 CD positive assay (culture, toxin A or toxin B) Treatment failure was defined as 2 consecutive days of diarrhea, and positive CD assay or pseudomembranes by endoscope at time of diarrhea, diarrhea no attributable to another cause	Probiotic 65.4% (17/26) Placebo 35.3% (12/34) p=0.04 Subjects treated for initial CD (n=64) Probiotic 81.3% (25/31) Placebo 75.5% (25/33) p=0.86		nausea, pain, or vomiting
Non-antibiotic intervention versus placebo					
Mogg, 1982[76] Br J Surg United Kingdom	48 patients on a single surgical unit with severe diarrhea after antibiotic treatment 48 entered study and 10 withdrew (7 on resin and 3 on placebo), 38 analyzed (79%) No information on age and gender. Diarrhea defined as ≥3 loose stools/d or more than 1 L of drainage from colostomy	Absorptive resin Colestipol 10 g every day mixed in fruit squash x 5 days (n=17) Placebo (sherbet) (n=21) Stool tested for CD cytotoxin at study start, on day 3 and last day of treatment (day 5) Outcome was defined as return of diarrheal stool to normal, (i.e., 2 solid	Colestipol: 3/12 (25%) Placebo: 3/14 (21%)		Allocation concealment: Possibly adequate (“identical placebo”) Blinding: not reported Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes Notes: CD cytotoxin was not present in all subjects reported as cases

Table 19. Nonantibiotic intervention for treatment of *C. difficile* (continued)

Study	Sample	Intervention/ Comparison and Method	Resolution of CDAD* (Diarrhea and CD toxin Positive Stool)	Other outcomes	Study Quality
	Previous placebo group (n=22) from prior study of vancomycin and placebo on same unit.	stools in 24 hours) in stools that were positive for *C. difficile* OR its toxin			No statistical testing Some historical controls included with concurrent controls CD treatment and prevention findings were combined (i.e., findings of subjects with *C. difficile* or its toxin in stool before receiving the intervention were combined with those who acquired these *during* the intervention

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile*

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
Primary Prevention						
Surawicz, 1989[77] Gastroenterology United States RCT	318 hospitalized patients given new antibiotics 180 completed study, 138 had *C. difficile* tested Mean age: 46.5 years Gender: Male 69%	Probiotic: *Sacchromyces boulardii* (250 mg capsule with 1 g S. boulardii bid (n=116) Placebo bid (n=64) Stools collected for CD culture at entry, day 5 then q 10 d, end of study, and when have diarrhea (diarrhea ≥3 loose/watery stools/s x 2 d); CD culture + stools were tested for cytotoxin; need 3 stools tested for CD and ≥8 days of monitoring for inclusion	Overall incidence of CDAD: Probiotic: 3/116 (2.6%) Placebo: 5/64 (7.8%) Fisher's Exact test performed by reviewers p=0.13 Incidence of diarrhea in 48 patients had stools that were CD toxin+: Probiotic: 3/32 (9.4%) Placebo: 5/16 (31%), test of significance between groups p=0.07			Allocation concealment: not defined Blinding: double Intention-to-treat analysis*: no, 138 not evaluated (43%) Withdrawals and dropouts adequately described: yes
McFarland, 1995[174] Am J Gastrenterol United States RCT	193 hospitalized adult patients receiving new beta-lactam antibiotic with or without another antibiotic and no diarrhea 129 (67%) completed study Mean age: 42 years Gender: Male 65%	Probiotic: Lyophilized *S. boulardii* 3x10^{10} cfu (1 g) orally in two 250 mg capsules/d within 72 hours of antibiotic and until max of 28 days (n=97) Placebo 1 g (undefined) (n=96) Followup for 7 days after stopping drug	Overall incidence of AAD: Probiotic: 3/97 (3.1%) Placebo: 4/96 (4.2%) Fisher's Exact test performed by reviewers p=0.72 Development of ADD in 24 patients with positive CD assays: Probiotic: 3/10 (30%) Placebo: 4/14 (29%) (The power of detecting a significant difference based on the sample size of 24 and the above rates was less than 3%)			Allocation concealment: adequate (appearance and odor of the capsules of interventions were identical, done centrally Blinding: double Intention-to-treat analysis: yes Withdrawals and dropouts adequately described: yes Notes: 3 reviewers of diarrhea
Lewis, 1998[78] J Infect United Kingdom	72 Hospitalized elderly (≥65 years) patients started on	Probiotic: *S. boulardii* (113 mg) (Ultra-Levure, Biocodex, Montrouge, FR) 2x/day	Overall incidence of CDAD: 4 patients had diarrhea		CD toxin only Probiotic:	Allocation concealment: adequate (pharmacy-

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile* (continued)

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
RCT	antibiotics Mean age (range): 74 (70-85) years Gender not reported ("no difference between sex")	(n=33) Placebo (undefined) 2x/day (n=36) Stool sample sent to lab every 4th day or if diarrhea (≥3 loose stools in 24 hours) to test for CD toxin	stools that were CD toxin+ (not reported by treatment arm) No statistically significant difference in diarrhea stools with + CD toxin between probiotic and placebo (data and percents not reported)		5/33 (15%) Placebo 3/36 (8%) No statistical testing done	controlled) Blinding: probably double, nursing staff blinded to treatment assignment Intention-to-treat analysis: no, 3 excluded Withdrawals and dropouts adequately described: yes
Thomas, 2001[79] Mayo Clinics United States RCT	302 hospitalized patients on antibiotics 267 (88%) completed study Mean age (range): 56 (18-93) years Gender: Male 54%	Probiotic: *Lactobacillus* GG (20×10^9 cfu + inulin filler) (CAG Functional Foods, Nebraska) 1 capsule 2x/d x 14 d (n=133) Placebo (inulin filler) (n=134)	<u>Overall incidence of CDAD:</u> Only 5 patients (1.9%) with positive CD toxin: Probiotic: 2/133 (1.5%) Placebo: 3/134 (2.2%), p >0.99		CD toxin in 1st 21 days after enrollment per retrospective chart review data of patients with CD at hospital Probiotic: 5/133 (4%) Placebo: 3/134 (2%), no statistical testing	Allocation concealment: adequate (pharmacy-controlled) Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes Notes: CD results by randomized chart review How test CD toxin not reported No association of diarrhea with CD toxin+
Plummer, 2004[80] International Microbiol United Kingdom RCT	150 elderly hospitalized patients started on antibiotics 138 (92%) completed study	Probiotic: *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, 2×10^{10} cfu in 1 capsule/d (Cultech, Saansea) for at least 20 of antibiotic therapy (n=69)	<u>CD diarrhea, 1st testing, during diarrhea and antibiotic tx in hospital:</u> Probiotic: 2/69 (3%) Placebo: 5/69 (7%), no statistical testing reported, Fisher's Exact test performed by reviewers			Allocation concealment: not defined Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile* (continued)

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
	Age and gender not reported	Placebo (n=69) Start therapy within 36 hours of antibiotics, probiotic = 1.12 days after antibiotics placebo = 1.10 day Stools were cultured for *C. difficile*, then if culture +, CD toxins A and B were tested - at start of study - if diarrhea - after antibiotics completed during hospitalization or discharge	p=0.44 Proportion developing diarrhea positive for *C. difficile*-associated toxins was 4.35% lower in probiotic group (95% CI of –0.132 to 0.038). <u>CD diarrhea, 2nd testing of same patients after antibiotics completed or at discharge:</u> Probiotic: 2/69 (3%) Placebo: 6/69 (9%)			described: no Notes: Diarrhea is undefined Study participation stopped if on antibiotics >20 days
Lewis, 2005[83] Aliment Pharmacol Ther United Kingdom RCT	450 hospital patients ≥65 years prescribed a broad spectrum antibiotic within past 24 hours 15 (3.3%) patients withdrew or were withdrawn from study N=435 who finished Mean age (range)::77 (70-84) years Gender: Male 49%	Prebiotic: Oligofructose (12 g/d) (n=215) Placebo (sucrose 12 g/day) (n=220) Taken during antiobiotics + 7 days after Follow up for additional 7 days *C difficile* toxin was measured if diarrhea (1-2 loose stools/day)	54 (12%) patients were culture-positive for *C. difficile* on study entry. Prebiotic: 19/213 (9%) Placebo: 21/220 (9.5%), no statistical testing reported, Fisher's Exact test performed by reviewers p=0.87			Allocation concealment: Unclear Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes Notes: Subjects were withdrawn from study if they experienced significant diarrhea (>3 stools/day) Stated intent to treat but some % are for N=433 not 435 or 450 who enrolled No increase in bloating, stool form or interval between stools by prebiotic

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile* (continued)

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
Can, 2006 [81] Med Sci Monit Turkey RCT	151 adult inpatients between 25-50 years who had chemo-therapy and antibiotics Gender: Male 95%	Probiotic: *S. boulardii* + antibiotics (β lactam) (n=73) Placebo + antibiotics(n=78) 2x/day started 48 hours or less after antibiotic therapy started for duration of antibiotic tx CD toxin A tested in those with diarrhea	<u>Overall incidence of CDAD:</u> 8 patients had diarrhea, only two CD toxin + (both in the placebo group Probiotic: 0/73 (0%) Placebo: 2/78 (2.6%) Fisher's Exact test performed by reviewers p=0.50			Allocation concealment: not defined Blinding: double Intention-to-treat analysis: yes Withdrawals and dropouts adequately described: none reported Notes: Duration of probiotic may have been variable Diarrhea is undefined
Hickson, 2007[82] BMJ RCT	135 hospital patients taking antibiotics were given tx until tx was finished + 1 week; If discharged from hospital and stayed on antibiotics, they continued tx; CD testing and followup occurred for 4 weeks after tx ended 11/2002-1/2005 22 (16%) patients lost to followup and not included in the analyses 12 in probiotic group and 10 in placebo 4 pts not tested for CD (1 on probiotics and 3 on placebo) Mean age: 74 years Gender: Male 46%	Probiotic: *L. casei* DN-114 001 (L casei imunitass, 1 x 10^8 cfu/ml) + *S.thermophilus* (1 x 10^8 cfu/ml) + *L. bulgaris* (1 x 107 cfu/ml) in yogurt drink (Actimel, Danone, FR) (n=69) Placebo: sterile milkshake (Yazoo, Campina NE) (n=66) Drinks consumed during antibiotics therapy + 1 week CD toxins A and B tested in diarrheal stools CD diarrhea = CD toxins A and/or B and diarrhea stools (2 liquid stools/d x 3 or more days in an amount greater than normal for the patient)	<u>Overall incidence of CDAD:</u> Probiotic: 0/57 Placebo: 9/53 (17%), p=0.001 Absolute risk reduction = 17% (95% CI 7% to 27%) NNT = 6 (4 to 14)			Allocation concealment: adequate (pharmacy - controlled) Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes Notes: Good compliance with drink, 75% for probiotic and 79% placebo Type CD toxin not specified No adverse events from either drink

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile* (continued)

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
Prevent Recurrent CD Diarrhea						
Surawicz, 2000[71] Cl Infectious Diseases United States RCT	168 randomized adult inpatients and outpatients, 32 with recurrent CD disease *CD disease subjects* Age: 61.6 Gender: Male 41% *All subjects* Mean age: 63.2 years Gender: Male 29%	*CD disease subjects* Probiotic: *S. boulardii* (1 g/d) + high dose oral vancomycin (2g/d) (n=16) Placebo (1 g/d) + high dose oral vancomycin (2g/d) (n=16) Probiotic/placebo started on day 7 - day 28 CD disease = diarrhea + (≥3 loose/watery stools/s x 2 days or >8 loose stools/day within 48 hours) and positive CD assay (culture then toxin A or B) measured at multiple time points		Recurrence: Probiotic: 3/18 (17%) Placebo: 7/14 (50%), p=0.05		Allocation concealment: adequate, centralized Blinding: double Intention-to-treat analysis: yes Withdrawals and dropouts adequately described: none reported
Wullt, 2003[72] Scan J infect Dis RCT	29 adult patients from 9 centers with + CD toxin assay within 6 days of enrollment at least 1 prior episode CD diarrhea within past 2 months. And ongoing diarrhea 8 patients (28%) lost to followup were not included in analysis, 21 completed trial Mean age: 63.8 years Gender: Male 5%	Probiotic: *L. plantarum* in fruit drink with oats fermented by L. plantarum 299v (5 x 10^{10} cfu) x 38 days and Metronidazole (400 mg tid po) x 10 days Metronidazole + placebo fruit drink with chemically acidified oats	<u>Clinical cure:</u> no diarrhea (≥ 3 loose stools x 2 days) on days 5-10 of tx Probiotic: 11/12 (92%) Placebo: 9/9 (100%)	<u>Total recurrences:</u> Probiotic: 4/11 (36%) Placebo: 6/9 no statistical testing reported, Fisher's Exact test performed by reviewers p=0.37	<u>CD toxin negative</u>: Probiotic:7/12 (58%) Placebo: 5/9 (56%), p=.642	Allocation concealment: Not defined Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts adequately described: yes

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile* (continued)

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
Lewis, 2005[84] Clin Gastroenterol Hepatol RCT	142 consecutive elderly (≥65 years) in patients with *C. difficile* diarrhea and treated by their physician with metrondiazole or vancomycin for 10 days (if treatment failure (diarrhea and CD toxin A and B) or intolerance with metronidazole). Mean age: 75 years Gender: Male 58%	Prebiotic: Oligofructose (12 g/d) (n=72) Placebo (sucrose 12 g/day) (n=70) Taken as soon as possible after dx of CD diarrhea and + 30 days after CD diarrhea = 3 loose stools in 1 day and + CD toxin		Relapse of diarrhea (CD was not retested) Prebiotic: 6/72 (8.3%) Placebo: 24/70 (34.3%), p<0.0001		Allocation concealment: Not defined Blinding: single Intention-to-treat analysis: no, 1 was lost to followup and 8 never resolved diarrhea to be able to relapse and were excluded Withdrawals and dropouts adequately described: yes Notes: CD was not measured at diarrhea recurrence
Lowy, 2010[85] N Eng J Med United States and Canada RCT	200 inpatients and outpatients were ≥18 years of age with diarrhea associated with a positive stool test for *C. difficile* toxin(s) in the 14 days prior to enrollment and who were receiving either metronidazole or vancomycin Mean age (range): 64 (20-101) years Gender: Male 34% White race: 88% Black race: 6% Severe disease at enrollment: 40%	Monoclonal antibodies: intravenous infusion of fully human monoclonal antibodies against *C. difficile* toxins A (CDA1) and B (CDB1) x 1 (n=101) Placebo (0.9% sodium chloride) x 1 (n=99) Recurrence of *C. difficile* infection, was defined as a new episode of diarrhea associated with a new positive stool toxin test after the resolution of the initial CDI diarrheal episode and after discontinuation of metronidazole or vancomycin. Diarrhea was defined as three or more unformed		Incidence of laboratory documented CDAD - primary outcome): Monoclonal antibodies: 7/101 (7%); inpatient = 7/50, outpatient = 0/51 Placebo: 25/99 (25%); inpatient = 13/52, outpatient = 12/47, p=0.0004 Recurrent diarrhea With/without laboratory		Allocation concealment: not defined Blinding: double and independent statistician and data and safety monitoring board Intention-to-treat analysis: yes Withdrawals and dropouts adequately described: yes Adverse events reported in 14 patients (antibody group = 9; placebo = 5) during infusion and in 11 patients (antibody group = 6; placebo = 5) during 2-hour period after infusion.

Table 20. Probiotic or prebiotic interventions for prevention of *C. difficile* (continued)

Study/ Design	Sample	Intervention/ Comparison and Method	*C. difficile* diarrhea (Diarrhea and CD Toxin +)	Later Recurrence of CD Diarrhea	CD Toxin+	Notes of Study Quality and Side Effects
		stools per day for at least 2 consecutive days or more than six unformed stools in 1 day		confirmation of *C. difficile* infection and with/without antibiotic treatment for *C. difficile* infection Monoclonal antibodies: 28/101 (28%) Placebo: 49/99 (50%), p=0.0022		All AE noted to be mild to moderate (headache reported most frequently) Death: antibody group = 7; placebo = 8 (p=0.79)

Table 21. Case studies/series and potential harms of nonantibiotic treatments for *C. difficile*

Study/ Study Focus	Subject/Study Details	Interventions	Clinical Diarrhea Outcomes	CD Toxin, CD Culture, or Other	Adverse Effects (AE) Harms
McDonald, 1984[163] Treatment	Male, aged 76 years Tx for diarrhea and PSC per sigmoidoscopy	Choleystyramine (toxin absorbing resin) 12 grams(g)/day (d)orally	PMC resolved at autopsy	CD toxin + stools for 1 month after symptomatic relief CD culture + stools for 1 month	Choleystyramine particles in arterial and venous vessel walls at autopsy Ulcerated esophagus was likely portal
Kunimoto, 1986[162] Treatment	Female, aged 38 years with PMC and CD cytotoxin and 4 recurrences of symptoms after vancomycin and metronidazole	Choleystyramine 12 day/orally x 12 months.	"Rapid symptoms relief"		Not reported
Kimura, 2007[175] Treatment	2 men, aged 71 and 54 years with PMC by colonoscopy, CD toxin A and septic shock	IV "hemoperfusion" agent: Vancomycin (2 g/d) orally + polymixin B-immobolized fiber 80-100 mg/d IV x 7-14 days		CD toxin became negative after 7 days PMC resolved after 7 days	Not reported
Peppe, 2008[176] Phase 1 dosing study and safety	40 healthy male volunteers, mean age 47 (SD 20) years 10 cohorts of 8 patients received treatment and 2 received placebo in each group	Tolevamer (nonantibiotic binder of CD toxins A and B) Sequential dose escalation 9,12 and 15 grams/day x 9 d	Not available	Not available	68 mild AEs 14 in placebo group (abdominal distension, pain, defecation urgency, flatus, loose stools) In 15 g/d group, decrease in 24 hour urinary potassium excretion averaging 12 mmol/24 hours vs. baseline
Tvede, 1989[86] Treatment	6 patients with chronic relapsing CD diarrhea 1 male and 5 females, aged 6 - 72 years 6 previously treated with vancomycin, 1 treated	Fecal transplant: enema of fresh feces from a healthy relative 1 patient Enema of mixture of 10 strains of bacteria: E.		All had stools negative for CD toxin after enema and at 1 yr followup All had stools negative for CD culture after enema and at 1 yr	Not reported

Table 21. Case studies/series and potential harms of nonantibiotic treatments for *C. difficile* (continued)

Study/ Study Focus	Subject/Study Details	Interventions	Clinical Diarrhea Outcomes	CD Toxin, CD Culture, or Other	Adverse Effects (AE) Harms
	with cholestyramine and vancomycin and 5 were treated with metronidazole also	coli (1109 & 1108-1), *Cl innocuum, Cl ramosum, Bact. Ovatus, Bact. vulgatus, Cl bifermentans, Bact. Thetaiotao-micron, Peptostrepto-coccus productus, Cl bifermentans*		followup	
Persky 2000[87] Treatment	Female, aged 60 years who failed vancomycin treatment of CD toxin+ and diarrhea	Fecal transplant: enema of fresh feces from a healthy relative 1 patient	Diarrhea resolved	Stools negative for CD toxin	Not reported
MacGregor, 2002[164] Treatment	Female, aged 42 years with multiple health problems then respiratory failure and critically-ill Developed CD toxin and diarrhea Failed metronidazole switched to vancomycin and yogurt added	Probiotic: yogurt with live cultures (supermarket brand) + Vancomycin			*S. pneumonaie* and secondary *L. rhamnosus* septicemia and death
Pakyz, 2007[177] Treatment	Female, 87 years of age, with recurrent diarrhea and C difficile antigen in stool	Probiotic: lactinex (lactobacillus) 1 g orally 3x/day + Metronidazole	Loose watery stools continued for 5 days	CD antigen in stools remained even with symptomatic relief on vancomycin. Switched to oral vancomycin with symptomatic relief	Not reported
Munoz, 2005[172] Treatment	Case studies + Retrospective chart review of ICU pts + review of 57 patients literature review. 3 ICU patients 3 ICU patients (females in 70s) with *S. cerevisiae* fungemia	Probiotic: 3 cases were treated with Ultralevura for CD diarrhea 2/41 patients without fungemia received probiotic		Probiotic cultures grew heavy yeast (>1 million cfu/ml) Mortality: 28% (17/60) (	60 patients total with fungemia, 28/47 (60%) in ICU, 26 (46%) were treated with the probiotic

Table 21. Case studies/series and potential harms of nonantibiotic treatments for *C. difficile* (continued)

Study/ Study Focus	Subject/Study Details	Interventions	Clinical Diarrhea Outcomes	CD Toxin, CD Culture, or Other	Adverse Effects (AE) Harms
	Charts of 41 (with 14 ICU) patients over 1 month of fungemia outbreak were reviewed				
Seal, 1987[178] Treatment	2 patients, aged 88 and 76 years, with diarrhea and CD toxin who failed antibiotic treatment.	Nontoxigenic strain of *C. difficile* (M-I) previously shown to protect hamsters One ml was suspended in 50 ml of milk to yield ~10 7 CFU *of C. difficile/*ml. *G*iven as a single oral dose on 3 successive days.	Symptoms decreased in 2 patients and CD toxin was (1) eventually 1 patient had a relapse of CD toxin and diarrhea x 2d 17 d after improvement		Constipation in 1 patient
Taylor, 2008[166] Phase 2 safety study	30 subjects, mean age 27.5 years (range 20-53) 33% male 5 cohorts of 6 subjects each	Monoclonal antibody to CD toxin A (CDAI)			No serious AEs possibly, probably or definitely associated to CDAI 3 moderate severity AEs (low BP, diarrhea) 18 mild severity (headache, nausea, loose stools, abdominal discomfort, BP changes)
Louie, 2009[169] Treatment, Phase II dosing study	48 Adults with ≥3 liquid or unformed stools/d or ≥6 diarrheal stools in 36 hours for a first relapse and treatment with vancomycin or metronidazole <24 hours CD culture and toxin tested on those who failed to respond and required further treatment.	Bactericidal Fermentation product: OPT-80 (18 member macrocycle) 50 mg q 12 hours x 10 d (total = 100 mg/d) (n=14) 100 mg q 12 hours x 10 days (total = 200 md/d) (n=15) 200 mg orally q 12 hours x 10 d (total = 400	Resolution of diarrhea within 10 day tx period: 1. 100 mg/d: 71% (10/14), 2. 200 mg/d: 81% (12/15) 3. 400 mg/d: 94% (15/16) Resolution of diarrhea after treatment with no further treatments: 1. 100 mg/day: 14% (2/14) 2. 200 mg/day: 7%	Recurrence of CD infection 1 mo. after treatment: 100 mg/day: 8.3% (1/14) Clinical failure: 400 mg/day: 6.3% (1/16) Need change of treatment: 1. 100 mg/day: 14% (2/14) 2. 200 mg/day: 13% (2/15) 3. 400 mg/day: 0	

Table 21. Case studies/series and potential harms of nonantibiotic treatments for *C. difficile* (continued)

Study/ Study Focus	Subject/Study Details	Interventions	Clinical Diarrhea Outcomes	CD Toxin, CD Culture, or Other	Adverse Effects (AE) Harms
	Resolution of CD infection (CDI): ≤3 BMS of any consistency/d without any other symptoms within 10 days of treatment and not requiring other treatment	mg/day) (n=16)	(1/15) 3. 400 mg/day: 6% (1/16)		
Sougioultzis, 2005[170] Treatment CD Vaccine	Subject 1: Male, 51 years of age. Subject 2: Female, 71 years of age. Subject 3: Female, 33 years of age. All had ≥3 unformed bowel movements per day for ≥2 days, associated with a positive stool toxin test for *C. difficile* (either tissue culture cytotoxin assay or toxin A or B enzyme immunoassay) that occurred within 30 days of discontinuation of therapy with metronidazole or oral vancomycin that had been administered for treatment of a prior episode of CD diarrhea.	*C difficile* toxoid vaccine form purified toxins A and B, injected IM at the deltoid region on 4 occasions, on days 0, 7, 28, and 56.		Subjects discontinued treatment with oral vancomycin after their fourth and final inoculation with the *C difficile* toxoid vaccine One of the 3 subjects did not show increase in serum antitoxin antibodies or serum toxin neutralizing activity	No AE that were definitely or probably related to vaccination. Adverse events that were possibly related to vaccination included a mild headache (subject 1) and mild abdominal pain (subject 2). Subject 2 also reported transient polyarthralgia after the fourth inoculation and later developed atypical polyarthritis with a normal erythrocyte sedimentation rate, a negative rheumatoid factor test, and slightly elevated C-reactive protein. Approximately 2 months after completion of the study, a clinical diagnosis of polymyalgia rheumatica was made by a rheumatologist
Kotloff, 2001[179] Randomized, double-blind, Phase 1 study of CD vaccine	30 healthy adults (no ages reported)	Vaccine, 6.25, 25 or 100 ug given IM Five subjects at each dose level received soluble toxoid vaccine, and five subjects			No serious adverse events during the study: rash = 8 (subjects); abdominal pain = 6; athralgia = 2; diarrhea = 2

Table 21. Case studies/series and potential harms of nonantibiotic treatments for *C. difficile* (continued)

Study/ Study Focus	Subject/Study Details	Interventions	Clinical Diarrhea Outcomes	CD Toxin, CD Culture, or Other	Adverse Effects (AE) Harms
		received an equivalent dose of toxoid adsorbed to alum.			All subjects had local pain at injection site, especially those who received toxoid adsorbed to alum, pruritus (without urticaria) at the site of injection n=6

Chapter 4. Summary and Discussion

There is very limited high quality evidence, to support the diagnostic, preventive, and treatment practices for CDAD carried out by providers in hospital, long-term care, and outpatient settings. Inconsistency in definitions of diarrhea, severity, and resolution of symptoms contributes to the difficulty in drawing conclusions from the evidence. Table 22 provides a summary of the evidence and results presented in this review.

Diagnostic Testing (Key Question 1)

This review focused on comparing the sensitivity and specificity of diagnostic methods that rely, at least in part, on the most widely used commercial immunoassays for *C. difficile* toxin. Immunoassays that can detect both toxins A and B were of particular interest because hypothetically they are the most sensitive immunoassay for detecting toxins and they are very commonly used by laboratories in the United States. In addition, data from comparative evaluations that included newer commercial *C difficile* toxin gene detection tests were of interest.

In general, there's little evidence that the sensitivities of commonly used immunoassays for toxins A and B differ, and any differences in their percent of false positives (1 – specificity) most likely are small (3 percent or less). However, the strength of the evidence is low due to the number of immunoassay comparisons that are available in the literature. The possibility exists that future research could impact the findings. Further, one article with eight comparisons is the sole source for many of the comparisons. The available comparative data doesn't rule out the possibility of larger differences between some of the immunoassays that have or have not been directly compared in adequate numbers. While the precision of the findings is such that we cannot rule out the possibility of differences in sensitivity on the order of three to five percent, it is unclear whether such differences would affect clinical decisionmaking.

Gene-based tests that used toxin B gene fragments tended to have better sensitivity than immunoassays for toxins A and B. Results, however, should be viewed with caution. Few studies contributed to the findings, and many direct comparisons were not found. Further, as mentioned above, the methodological differences between studies, including use of different reference tests, might have affected the toxin immunoassays more than the gene detection tests. Perhaps variation in the stability of the toxins in stool specimens as they were collected, stored, and processed contributed to the observed variation between studies in the estimates of the immunoassays sensitivities, whereas detection via amplification of gene fragments could be less susceptible to specimen degradation.

One comparison of a combined test that used a test for GDH followed by immunoassays for toxins A and B to the immunoassays for toxin A and B alone did indicate that the combined test had fewer false positives, as expected. However, the difference in sensitivity was imprecisely estimated and suggested that the combined test might be less sensitive, as expected.

These tests require varying skills, equipment, and time to carry out, and heterogeneity is a significant factor in reviewing the literature. Previous reviews by Planche et al.[88] and Crobach et al.[89] encountered difficulty comparing the sensitivities and specificities of the diagnostic tests in large part because there was too much variation between studies in the estimates of sensitivity and specificity of a particular test. Planche et al. used logistic regression with dummy variables to represent each immunoassay and found significant differences in sensitivity and specificity;

however, the regression model did not include any covariates to try to account for the substantial heterogeneity in the estimates between studies. We attempted to control for the heterogeneity between studies by examining the differences in sensitivity and specificity in stool samples tested within the same lab and did not find strong evidence of differences between tests within several immunoassays for toxins type A and B. The extent of any publication bias for these comparisons is unknown.

A clinically important question is whether the potential differences in the accuracy of the diagnostic tests being employed in practice would translate into differences in clinical behaviors or patient outcomes. Indeed, how well clinicians actually know the sensitivity and specificity of the test(s) for toxigenic CDI employed by their laboratories and incorporate this information into their patient care decisions is not clear.

Prevention (Key Question 2)

Very little evidence connects prevention strategies and techniques directly to patient related outcomes, such as CDAD incidence. Available evidence is generally from before/after study designs or limited time series. Hospital settings with outbreaks or hyperendemic episodes further limit applicability of the findings, and leave open the question of the relative contribution of regression to the mean (i.e., that CDAD rates returned to baseline rates even in the absence of effective interventions). The studies also varied in the degree to which they described CDAD surveillance, diagnostic accuracy, or laboratory performance. In most, surveillance was passive and depended on a positive toxin test on a stool specimen sent by clinicians caring for a patient with diarrhea. Unknown numbers of cases might have been missed or misdiagnosed. Additionally, attention has not been given to describing a prevention strategy's potential harm (e.g., increase in other pathogens, reduction in direct patient care contact due to isolation or restrictive contact requirements, increased costs) or the long-term sustainability of a practice.

There is low quality evidence that antibiotic prescribing practices appear to reduce CDAD incidence, a finding consistent with the Cochrane review.[34] None of the studies explicitly addressed the potential harms of changes in antibiotic use policy, but there are several theoretical harms. They include the possibility that preferred drugs will be less effective than drugs that physicians are discouraged from using, or drugs that are made unavailable for treating infections other than CDAD. Preferred antimicrobials might have greater costs or greater toxicities unrelated to CDAD. *C. difficile* strains might evolve to develop resistance to the preferred antibiotics, which might increase the likelihood that the recommended antibiotics might induce CDAD.

While several studies found increased risk with specific antibiotics or antibiotic classes, the antibiotics that confer greater risk for CDAD have changed over time and vary by location because of differences in prevalent toxigenic strains and especially the susceptibility patterns of those strains.[90] Clindamycin resistance was identified soon after the role of *C. difficile* in pathogenesis was discovered.[39,91,92] More recently, quinolones have assumed greater importance because strains have become more resistant over time.[93]

Fewer studies are available to support prevention practices aimed at breaking transmission. There was limited low quality evidence that gloves, disposable thermometers, handwashing, and intensive disinfection solutions help to reduce CDAD incidence. In addition, the presence and use of alcohol gel to prevent other hospital acquired infections, such as MRSA, did not increase

the rate of CDAD incidence, suggesting the possibility that providers and hospital staff did not substitute alcohol gel for hand washing.

Similar to the antibiotic prescribing practice research, none of the studies aimed at breaking transmission addressed potential harms for other prevention practices. Costs of disinfection, time to perform disinfection, and the possible harm to surfaces and equipment should be anticipated. Failures with vapor disinfection systems would be possible and might lead to toxic exposures of personnel or patients. Nor is there evidence to inform infection control professionals whether such practices are sustainable after an intervention period. That is, we cannot answer whether environmental cleaning staff will have developed professional habits that will continue when the intense monitoring related to an intervention period discontinues.

The potential for prevention research is often compromised by the swift uptake of newly described prevention strategies with the belief that these will improve institutional practices, health care quality, and reduce CDAD morbidity and mortality. Current prevention strategies often rely on studies using intermediate outcomes such as process. Newly acquired strategies are then added to current practice, bundling them into multiple component interventions. Multiple component prevention strategies document the multipronged prevention efforts in natural settings and have been reported in the literature to be successful in response to outbreak or hyper-endemic situations. It is very difficult, if not often impossible, to tease out the relative contributions of single components to the overall multiple component bundle of prevention strategies.

The realities of the environment and the habits of people who occupy those environments are complicated, and care must be taken to avoid assuming the effectiveness of preventive practices based on apparent logic. For example, hand washing is a logical and simple sounding strategy. However, if handwashing is performed using the same facilities as the patient, depending on the state of cleanliness in the room, handwashing may be negated the moment a surface is touched by the freshly washed hands. Further, *C. difficile* spores may persist for up to 5 months on some surfaces.[180] This very issue of complexity is in part what drives the aforementioned practice of adding prevention components with what might, under other conditions, be considered minimal available evidence.

Standard Treatment (Key Question 3)

The available evidence is insufficient to say whether any antimicrobial treatment is better than another, including the two most commonly used treatments, metronidazole and vancomycin. Our results build upon, and are consistent with, the Cochrane Review completed by Bricker et al.[181]

The total number of subjects from comparative studies on metronidazole and vancomycin is just 335 patients. This raises the possibility that, although a significant difference in effectiveness has not been detected, a true difference may exist. There is also no evidence for a difference in effectiveness for other agents, but again the possibility remains that such a difference exists. However, at this time any claims that one agent is superior to another for all cases of CDAD are not supported by available evidence. The findings apply to general adult inpatients. Bias due to selectively reporting outcomes is possible if cut-points are changed for CDAD definitions, e.g., number or consistency of stools. The clinical differences of changes in cut-points are also unknown, however, so the clinical significance could remain.

We found limited low-quality evidence that vancomycin was superior to metronidazole for subjects classified as having severe disease. This finding came from a sub-group analysis of a single trial; thus, confirmation is needed. Strengthening this finding is the fact that it was a pre-specified analysis, and that the severity classification appears to have been made before treatment allocation. An argument can be made that even small increases in effect size are important to a high risk patient population where time is of the essence.[94]

Outcome definitions varied significantly. For the assessment of initial cure, methods varied between only assessing symptoms, to a combination of symptoms and either laboratory values (stool studies, C-reactive protein levels, peripheral blood leukocyte count) or a physiologic measure (temperature). Similarly, definitions of resolved diarrhea ranged from greater than three formed stools in a 24 hour period [62] to greater than two formed stools daily[68] to "no loose stools."[65] Assessment of recurrence in these studies was similarly complicated by variable definitions, followup periods (21 days in two, 30 days in the other), and lack of detail regarding whether active or passive surveillance was used to detect recurrence.

None of the RCT studies included data regarding any other organisms, either with regard to colonization or subsequent clinical infection. Selection of VRE in particular has long been a concern when treating CDAD; usually this concern has involved the use of vancomycin, but increasingly it has been recognized that other antimicrobials can also select for increased rates of VRE carriage.

Nonstandard Treatment (Key Question 4)

We sought to document the range of treatments under investigation for treatment and prevention of CDAD, particularly recurrent CDAD. Overall, definitions of CDAD, interventions and measured outcomes are variable across the nonstandard prevention and treatment literature.

In attempts to expand treatment options for high rates of treatment nonresponse, treatment failure,[182] relapse, and recurrence, researchers and clinicians are examining a number of potential lines of treatment options. The evidence for effectiveness of nonantibiotic interventions for treating CDAD shows that probiotics, prebiotics, tolevamer, *C.* difficile immune whey, and colestipol are not more effective in treating CDAD than standard antibiotic treatment with oral vancomycin or metronidazole or compared with placebo. The evidence supporting this conclusion is limited and of low quality. Administration of a probiotic to treat CDAD in critically ill patients increases risk for greater morbidity and mortality from fungemia without any established benefit.

Prevention of CDAD, both initial and recurrent cases, through interventions intended to improve gut flora and host immunity is also a very active topic in the literature. Indeed, the majority of ongoing trials accessed through ClinicalTrials.gov are of this type of research. (See the Future Research Needs section and Table 23 following this section.)

There is limited, low quality evidence that the nonantibiotic prevention interventions are not more effective than placebo for primary prevention of CDAD. There is limited evidence of moderate quality that administering the prebiotic oligofructose or a monoclonal antibody to *C. difficile* toxins A and B along with standard antibiotics for CDAD are better than placebo and active control in preventing recurrence of CDAD in patients treated for CDAD. Although the studies for both treatment and prevention of CDAD using a nonantibiotic intervention included components of experimental designs, few had adequate rigor to yield high quality findings or

power to detect a significant difference between the interventions (or placebo) compared. In some studies, a low rate of CDAD precluded statistical testing.

There were five systematic reviews[183-186] and one meta-analysis[187] about the effectiveness of probiotics for treating CDAD, one systematic review about immunoglobulin treatment of CDAD,[188] and one systematic review included studies about prevention of CDAD using probiotics[186] (Appendix Table C11).

The five systematic reviews on the effectiveness of probiotics for treating CDAD included between three and six studies. The authors of the systematic reviews determined that the variability in methods such as types of probiotics, outcome measures, and types of subjects did not support pooling estimates in a meta-analysis. The conclusions of the systematic reviews were that there was no evidence (two reviews), sparse evidence (two reviews,) and insufficient evidence (one review) of any benefit of probiotics for CDAD. The systematic review on probiotics that also addressed prevention of CDAD included only one prevention study and concluded there was sparse evidence supporting this use of probiotics. The one meta-analysis of using probiotics for CDAD included six studies[187] and has been met with criticism. The criticism noted, among other points, the combination of findings from studies of treatment and prevention of adults and children, conducting a pooled analysis on results from heterogeneous outcome measures, analysis of results of studies with low quality due to flawed designs or methods, and lack of independent review as the investigator reviewed their own studies. The conclusion from the meta-analysis was that there was benefit in using a probiotic, especially one containing *S. boulardii*, for reducing recurrence of CDAD. The one systematic review of immunoglobulin was based on four retrospective studies and five case reports and concluded no recommendations could be made.[188]

Caution is recommended regarding new, nonstandard treatments, and not extrapolating study findings beyond the data. For example, one cannot assume if a probiotic treatment is effective for Antibiotic Associated Diarrhea that it will be effective for CDAD. Likewise, attention should be paid to which patients were included and excluded in probiotic treatment studies. Such studies generally exclude high-risk patients. Thus, there is no evidence for the use of probiotics in high risk patients. During a TEP call it was noted that some ICUs have banned probiotics in order to avoid the potential for fungemia and bacteremia in very ill patients.

Future Research

There are a number of important questions to be addressed regarding: (1) how to control, both infections and outbreaks; (2) what to do to prevent transmission after occurrence; (3) how to diagnose; (4) how to treat; and (5) how to change prevention strategies with outbreaks. Table 24 provides a summary of the research recommendations.

Diagnostic tests. It is difficult to apply the available evidence from comparative studies to help select the best diagnostic test(s) for clinical applications. Estimates of sensitivity and specificity for many diagnostics tests for toxigenic CDI can vary greatly between laboratories. Further, there is no high grade evidence from direct, within laboratory, comparisons of the performance of currently available diagnostic tests. The reviewed comparative studies did not clearly define the testing scenario including the setting, disease prevalence, patient selection criteria, patient characteristics, or the signs and symptoms of the suspected CDAD, making it difficult to judge to whom the study results might apply. Ultimately, the clinical importance of estimated differences in sensitivity (true positives), false positives, specificity (true negatives)

and false negatives, depends on how these types of test results would affect clinical decisions and patient outcomes.[189]

More research is needed to understand how test sensitivities and specificities are used to make decisions in clinical practice, and to define clinically meaningful differences based on their effects on clinical decisions and patient outcomes. Multicenter studies that (1) consistently use the most clinically relevant reference test, (2) use explicit clinical criteria to select patients and stool specimens to be tested, (3) randomly assign patients to different diagnostic tests, and (5) use key clinical outcomes as study endpoints are needed to fill this major gap in knowledge about diagnostic tests for toxigenic *C. difficile*.

Whether the newer gene amplification and detection tests are more consistent across laboratories, and more sensitive than the currently used toxin immunoassays without substantial loss of specificity, needs further study. Most importantly, studies are needed to demonstrate that use of tests that detect genetic residue related to *C. difficile* toxin production rather than the toxins *per se* lead to better patient outcomes.

Prevention. A number of potential prevention strategies can, and should, be investigated as a single intervention in a controlled trial in order to understand its potential contribution to a prevention program. However, the main obstacle to research in this area is the contextual setting.

Prevention happens within an institutional environment. It happens as a comprehensive approach for multiple potential hospital acquired infectious agents and attending to multiple potential vectors of transmission and host susceptibility. Researchers and decisionmakers may need to consider another approach to inform decisionmaking: a collaborative research process in which consensus agreements are reached for minimum data sets and followup periods, and definitions of interventions are agreed to in order to facilitate pooling data across organizations. Datasets of this nature could allow for employing more sophisticated epidemiological and decision analytic techniques to tease apart the relative contributions of different prevention strategies. The nature of the decisions faced by infection control professionals is qualitatively different than a physician's clinical decisions for an individual CDAD patient. Decision analytic techniques may be particularly valuable in this venue.

Standard treatment. The greatest needs for future studies for CDAD treatment are consistent definitions and reporting of outcomes, a uniform and clinically relevant definition of disease severity, and trials with adequate power to detect clinically meaningful differences in outcomes. In particular, trials need to include adequate numbers of subjects to allow stratification by patient characteristics such as age, gender, and comorbid conditions in order to address questions regarding the most effective therapy for CDAD. A well validated and clinically meaningful severity score would also assist in treatment decisions. Although most agents for CDAD appear to be well tolerated, explicit reporting of adverse events by treatment allocation is another area where future research can improve our understanding of optimal management of this disease.

Although identifying the strain of *C. difficile* is of great relevance to researchers, and can offer useful information to hospital epidemiologists, at present, strain identification is rarely performed in clinical settings. Thus, few clinicians treating CDAD are aware as to which strain of *C. difficile* is causing an individual patient's disease, and can at most make an assumption as to the strain type based on current epidemiology reported in the literature. This limitation makes any difference by strain in treatment efficacy of uncertain relevance.

Nonstandard treatment. Additional research on nonantibiotic interventions as adjunctive or alternatives to standard antibiotics for preventing and treating CDAD is needed and encouraged.

Studies to prevent recurrence of *C. difficile* are a priority of prevention. As no single approach has been shown to be superior, promoting studies of different types of interventions is reasonable at this time.

Fecal biomass transplant is one novel therapy for which continued research is supported. Findings of one fecal biomass transplant case show that the colonic microbial profile of the donor temporarily resembles that of the normal donor, which might explain its beneficial effect.[190] Guidelines related to screening for safety and selecting donors that would need to be considered in future studies have been outlined.[191] Of all the nonantibiotic interventions, probiotics have been investigated in the most studies, and the results are not encouraging. Unlike fecal transplant, probiotics provide only a single or few strains of bacteria, and thus may be insufficient to correct alterations in the complex and extensive micorbiome to the extent needed be therapeutic. The genomic mapping of indigenous microflora may offer new information to guide future formulation of a probiotic that can effectively target alterations in the microbiome in CDAD and other diseases of the colon.[192,193] A third strategy related to modifying microbial ecology in CDAD for which additional research is supported is administration of a nontoxigenic strain of *C. difficile*. Colonization with nontoxigenic *C. difficile* in hamsters protects against *C. difficile* disease after challenge with a toxigenic strain. The nontoxigenic strain of *C. difficile* is thought to directly compete with toxigenic strains of *C. difficile*.

Development of a binder of *C. difficile* toxins A and B, Tolevamer, appears to build on and refine earlier approaches using less specific absorptive resins, such as choleysteramine. Tolevamer was nevertheless inferior to standard antibiotic treatment. The results suggest that additional research characterizing the action of *C. difficile* toxin in the intestinal lumen might guide the design of a more effective anti-toxin agent. A vaccine to *C. difficile* toxin to bolster the immune response to the disease is an alternative systemic approach that should be developed further.

Studies are needed to determine whether some patients might be more likely to respond to nonantibiotic interventions. Sampling in current studies of nonantibiotic interventions varies considerably, ranging from individuals who are just starting antibiotics for infections other than *C. difficile*, those who have had multiple failures of antibiotic treatment for CDAD itself, and those who have had *C. difficile* in the past. Whether any one type of nonantibiotic intervention is effective in all of these types of cases is a question. More information is needed about patients who are at high risk for recurrence of CDAD.

The effects of sequencing therapies (antibiotic as well as nonantibiotic) on the resolution of CDAD merits further research. Studies show a variety of procedures for administering probiotics to prevent CDAD, for example, such as during standard antibiotic therapy or for a period after standard treatment is completed. Determining the optimal timing to introduce nonantibiotic interventions to possibly maximize their effect is recommended. For example, studies in hamsters indicate that timing of administration of a nontoxigenic strain of *C. difficile* for successful colonization as potential protection against disease differs depending on whether the animal is receiving antibiotics to which *C. difficile* is susceptible or resistant.[171]

Methodological Improvements. It is essential that future studies of a nonantibiotic intervention for treatment or prevention of CDAD be supported by a power analysis, adequate sample size, and an intent-to-treat analysis, in addition to other standard quality components of experimental design. Multi-center studies may be necessary to achieve adequate sample sizes. Laboratory confirmation of a pathogenic *C. difficile* organism (e.g., by toxin testing) and clinical symptoms of disease (e.g., diarrhea) are essential not only for study eligibility but for

determination of recurrence in long-term followup. Adoption of a standard definition of diarrhea as part of the definition of CDAD is strongly recommended. Similarly, a standard definition of CDAD resolution should be adopted. RCTs that compare more than one type of nonantibiotic intervention are suggested for efficiency.

On-going research. CDAD is an active area for research. Table 23 provides a list of 23 relevant ongoing or recently completed trials not yet published for the diagnosis, prevention, and treatment of CDAD. While there is considerable activity, none of the studies listed is expected to provide a definitive answer for any of the research needs discussed above.

Table 22. Summary of evidence

Key Questions	Level of Evidence	Summary/Conclusion/Comments
Key Question 1 Diagnostics		
Immunoassays for toxins A and B	Low to moderate	• Eight studies directly compared at least two immunoassays for toxins A and B, providing 15 pairwise comparisons of 7 difference immunoassays. Comparative data were not found for many currently used tests. • Precise estimates of the differences in sensitivity were not found nor were there any statistical differences between the tests that were compared. • Substantial differences in false positives, i.e. specificity, were not found among the tests that were compared.
Gene detection tests vs. immunoassays for toxins A and B	Low to moderate	• Three studies compared at least one gene detection test to at least one immunoassay for toxins A and B, providing a total of nine direct comparisons. Comparative data were not found for many currently available gene detection tests. • The gene detection tests can be substantially more sensitive than immunoassays for toxins A and B, with no or relatively modest loss of specificity.
2-step tests vs. immunoassays	Low	• One study compared at least one enzyme immunoassay for toxin A B to at least one two-step test using GDH as the first step and the toxin A and B immunoassay as the second step if the first was positive. • The two-step test produced fewer false positives (better specificity). However, the effect of the two-step test on sensitivity was estimated very imprecisely, but tended to be lower.
Patient characteristics	Insufficient	• Insufficient patient information for comparative data was found.
Key Question 2 Prevention		
Antibiotic use	Low	• Sixteen studies, including 6 bundled prevention practice studies, found appropriate prescribing practices are associated with decreased CDAD incidence • Harms were not reported.
Gloves	Low	• One controlled trial found use of gloves in hospital settings reduced CDAD incidence.
Disposable thermometer	Very low	• Three time series/before-after studies, two with controls, found use of disposable thermometers in hospital settings reduced CDAD incidence.
Handwashing/ alcohol gel	Very low	• No study examined whether handwashing reduced CDAD incidence. • Two studies, one controlled trial and one before/after study, of use alcohol gel to reduce MRSA transmission did not find significant differences in CDAD incidence
Disinfection	Very low	• Thirteen before-after studies of outbreaks or endemic hospital settings found intensive disinfection with a chemical compound that kills C. difficile spores reduced CDAD incidence.
Sustainability	Insufficient	• No evidence was available.
Risk factors	Very low to low	• Ten observational studies found evidence for antibiotic use, whether specific or general, increased risk of CDAD. • Severe underlying disease, acid suppression, and age are indicated as risk factors. A number of other potential factors may be indicated in single studies.
Multiple component strategies	Insufficient	• Eleven time series/before-after studies examined bundles of prevention components in a single intervention. Data is insufficient to draw conclusions.

Table 22. Summary of evidence (continued)

Key Questions	Level of Evidence	Summary/Conclusion/Comments
		• Harms were not reported.
Key Question 3 Antibiotic Treatment		
Vancomycin vs. metronidazole	Moderate for clinical cure, low for all other outcomes	• There were three head to head trials with a total of 335 subjects. Trials used various definitions of CDAD patient and cure definitions, especially with regard to stool count and consistency. • No significant differences in outcomes, including initial cure, clinical recurrence, mean days to resolved diarrhea, were found. • Our results build upon, and are consistent with, the Cochrane Review completed by Bricker et al.[181]
Severe disease, vancomycin vs. metronidazole	Low for clinical cure, very low for all other outcomes	• One RCT examined a pre-specified subgroup of 69 subjects with severe CDAD. • Vancomycin resulted in improved clinical cure, the equivalent of 203 more cases per 1000 subjects, or roughly two cases per ten subjects.
All other comparisons of standard treatments	Very low to low	• There were seven trials examining: vancomycin vs. bacitracin (two trials), vancomycin vs. nitazoxanide, vancomycin high vs. low dose, vancomycin vs. placebo, metronidazole vs. nitazoxanide, and metronidazole vs. metronidazole plus rifampin (one each).
Strain of organism	Insufficient	• No comparative data were available.
Patient characteristics	Insufficient	• No comparative data were available.
Resistance of other pathogens	Insufficient	• No data were available.
Key Question 4 Nonantibiotic Treatment		
Treating CDAD, active control	Low	• Probiotics, prebiotics, tolevamer, C. difficile immune whey, and colestipol, are not more effective in treating CDAD than standard antibiotic treatment with oral vancomycin or metronidazole or placebo.
Treating CDAD, placebo	Low	• Administration of a probiotic with live bacteria to treat CDAD in critically-ill patients increases risk for greater morbidity and mortality from fungemia without any known benefit.
Treating Recurrent CDAD	Very Low	• There is limited very low quality evidence from two case series that fecal biomass transplant is effective in treating recurrent CDAD for up to 1 year.
Preventing CDAD	Low	• There is limited evidence that the nonantibiotic interventions in this review are not more effective than placebo for primary prevention of CDAD.
Preventing recurrent CDAD	Low to moderate	• There is limited low quality evidence from one subgroup analysis that a prebiotic may reduce diarrhea recurrence in patients treated for CDAD more so than placebo with standard antibiotics. • There is limited moderate quality evidence from one study that monoclonal antibodies are effective in preventing recurrence of CDAD

Table 23. Trials from ClinicalTrials.gov and other sources

Intervention Comparator	Last Update, Estimated Completion	Sponsor	Study Design	Population	Primary Outcome	Code
Not yet recruiting – Diagnostic						
PCR, EIA, or cytotoxin assay	Feb 2010, 2010,	Hamilton Health Science, McMaster C. Lee, PI	Comparative	N=500 12+ years stool specimens	Test performance	NCT01066221
Not yet recruiting - Prevention						
I: Lactobacillus adicophilus/ rhamnosus complex C: placebo	Jan 2010, 2012	Vancouver Island Health Authority, C. Harder	RCT double blind	N=200 60+ patients on antibiotics	CDAD incidence	NCT01048567
I: Colostrum derived antibodies C: placebo	Aug 2009 2012	Hadassah Medical Organization	RCT double blind 60 day followup Phase II, III	N=300 18+ patients symptomatic patient, lab-confirmed CDAD	Recurrent CDAD, new cases in close proximity	NCT00747071
Recruiting - Prevention						
I: Saccharomyces Boulardii C:Placebo	July 2012	Bernhard Nocht Institute for Tropical Medicine S. Ehrhardt, PI	RCT double blind, Phase III	N=1,520 18+ hospitalized patients	CDAD incidence	NCT01143272
I: toxoid vaccine C: placebo	Apr 2011	Sanofi-Aventis	RCT double blind 9 week followup	N=650 18+ patients with one CDAD episode within last 10 days, not currently treated for recurrent	Recurrent CDAD	NCT00772343
I: Lactobacillus casei probiotic C: placebo	Apr 2010, 2012	University of Sussex, C. Rajkumar, PI	RCT double blind, 28 days, Phase II	N=1,200 55+ patients receiving antibiotics	Incidence and presence of toxin, prevent recurrence	NCT01087892
I: Clostridium butyricum MIYAIRI 588 Strain (CBM588 C: placebo)	Feb 2010, 2011	Osel, Inc. P. Lee, PI	RCT double blind, Phase II	N=200 18+ patients treated with vancomycin or metronidazole for CDAD	Recurrent CDAD	NCT01077245
I: Probiotic VSL#3 C: placebo	Sept 2009, Sept 2010	NHS, UK N. Haslam, PI	RCT double blind, 28 days, Phase II, III	N=450 18+ patients on antibiotics	CDAD incidence	NCT00973908
I: Recombinant human lactoferrin C: placebo	Sept 2006	John Hopkins W. Greenough, PI	RCT double blind	N=300 18+ LTC patients on enteral tube feeding	Not reported	NCT00377078

Table 23. Trials from ClinicalTrials.gov and other sources (continued)

Intervention Comparator	Last Update, Estimated Completion	Sponsor	Study Design	Population	Primary Outcome	Code
I: Hospital ward physical design C: traditional ward design	Mar 2009	University of Calgary W. Ghali, PI	Prospective controlled trial	N=3,600 18+ patients	CDAD incidence	NCT00563186
Recruiting - Treatment						
I: CB-183,315 two doses tested C: Vancomycin	Apr 2010, 2011	Cubist Pharmaceuticals	RCT double blinded, 3 arms, Phase II	N=210 18+ patients symptomatic patients, lab-confirmed, index or 1st recurrence	Not clear	NCT01085591
I: Loperamide (Imodium) C: placebo	Jan 2008, Dec 2009	VA Houston, D. Musher, PI	RCT double blind, Phase IV	N=120 18+ patients on antibiotics	Symptomatic treatment of diarrhea	NCT00591357
I: Nitazoxanide C: NA	Mar 2006	VA Houston, D. Musher, PI	Open label compassionate use Phase III	N=100 18+ patients with CDAD who failed therapy	Resolution of symptoms	NCT00304356
Fecal bacteriology	Mar 2006	VA Houston D. Musher, PI	Retro observational cross-sectional	N=80 18+ patients with CDAD, patients lab-confirmed without CDAD, patients who fail therapy, hospitalized patients on antibiotic with no diarrhea		NCT00304876
Completed - Prevention						
I: Bio-K+ CL-1285 probiotic, Lactobacillus acidophilus, Lactobacillus casei C: placebo	Aug 2009	Bio-K Plus International, Inc. G. XingWang, PI	RCT double blind, Phase III	N=255 50-70 years, patients on antibiotic therapy	CDAD incidence	NCT00958308
I: Lactobacillus adicophilus and casei C: placebo	Sept 2008	Bio-K Plus International, Inc. J. Dylewski, PI	RCT double blind	N=480 18+ patients on antibiotics	CDAD incidence	NCT00328263
I:Polycationic disinfectant C: alcohol-based gel and regular disinfectant with quat/chloramines	Aug 2008	Helsinki University Soft protector TEKES, M. Kanerva, PI	Open label, parallel active control 6 months	3 experimental wards plus control wards	CDAD incidence	NCT00566306

Table 23. Trials from ClinicalTrials.gov and other sources (continued)

Intervention Comparator	Last Update, Estimated Completion	Sponsor	Study Design	Population	Primary Outcome	Code
Completed - Treatment						
I:GT160-246 C: Vancomycin	Jul 2009	Genzyme	RCT double blinded, Phase II	N=300 18+ patient with mild to moderate CDAD	Not reported	NCT00034294
I: Rifaximin C: Vancomycin	Dec 2009	Salix Pharmaceuticals A. Shaw, PI	RCT double blind, Phase III	N=300 18+ lab confirmed CDAD	Resolution of baseline, recurrence	NCT00269399
I: OPT-80 C: Vancomycin	Feb 2010	Optimer Pharmaceuticals, YK Shue, PI	RCT double blind, Phase III	N=536 16+ patients with CDAD	Cure rate, recurrence rate	NCT00468728
I: Lactobacillus CL placebo (both arms used metronidazole)	Mar 2006	VA Houston D. Musher	RCT double blind Phase IV	N=70 18+ patients lab confirmed CDAD	Response to treatment	NCT00304863
I: Tolevamer potassium sodium C: Vancomycin, Metronidazole	Jul 2009	Genzyme	RCT double blind, 6 weeks	N=520 18+ patients with CDAD	Resolution of diarrhea	NCT00196794
Not ClinicalTrials.gov						
FECAL trial I: bacteriotherapy C: Standard antibiotic treatment		ZonMW, the Netherlands Organisation for Health Research and Development van Nood, PI	RCT, 10 week followup	N=120 18+ patients, proven relapse of CDAD. Exclude ICU, immuno-compromised	Response to treatment	Netherlands. Non-citizens accepted if travel to Amsterdam

Table 24. Future research recommendations

Key Question	Research Gaps	Types of Studies Needed to Answer Questions	Future Research Recommendation
Key Question 1. How do different methods for detection of toxigenic *C. difficile* compare in their sensitivity, specificity, and predictive values?	• Few comparisons are available. • Heterogeneity is an obstacle. • Unknown what differences in sensitivity and specificity would alter clinician decisionmaking • Unknown influence of patient and stool characteristics on test sensitivity and specificity	Comparison of diagnostic tests using same samples, same labs. Multicenter studies with well-documented patient samples.	• Document stool sample characteristics, patient selection criteria, patient characteristics, signs and symptoms of suspected CDAD.
Key Question 2. What are effective prevention strategies?	• Little evidence available with clinically important outcomes.	High quality comparative studies evaluating effectiveness and harms of single and/or multicomponent prevention strategies, including cleaning, isolation, antibiotic restriction Discrete simulation models	• Pool data from multiple participating hospital sites • Establish minimum data sets for observational data points which can inform models
Key Question 3. What are the comparative effectiveness and harms of different antibiotic treatments?	• Limited evidence available on whether vancomycin is more effective for severe CDAD.	High quality comparative studies with adequate power to detect significance in a priori subgroups	• A uniform and clinically relevant definition of severity • Subgroup analysis may include age, gender, comorbid conditions • Explicit reporting of adverse events
Key Question 4. What are the effectiveness and harms of nonantibiotic adjunctive interventions?	• Probiotics as a treatment adjuvant is not supported. Potential harms to seriously ill patients may outweigh potential benefits for further prevention research. • Probiotics as prevention warrants further study. • Further research of monoclonal antibodies for prevention is warranted • Further research of fecal transplant is warranted	High quality comparative studies with adequate power.	• Placebo comparators would contribute indirect evidence that would help guide potential combination therapies. • Quality research includes power analysis, intention to treat • Multicenter trials are likely needed to achieve adequate samples • Trials of probiotics for prevention are well represented in ongoing studies • Patient characteristics for sub-group analysis
Umbrella issues			• Adoption of standard definitions for diarrhea, CDAD resolution

References and Included Studies

(Note that there is a separate set of references at the end of the evidence tables in Appendix C and reference numbers are different than those in the text of the report).

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List of Acronyms and Abbreviations

AAD	Antibiotic associated disease
AE	Adverse effects
AHRQ	Agency for Healthcare Research and Quality
AMED	Allied and Complementary Medicine
CDAD	Clostridium difficile associated disease
CDI	Clostridium difficile infection
CI	Confidence interview
GDH	Glutamate dehydrogenase enzyme
GI	Gastrointestinal
GRADE	Grading of Recommendations, Assessment, Development, and Evaluation
ICU	Intensive care unit
LTC	Long-term care
MeSH	Medicine's Medical Subject Headings
MRSA	Methicillin-resistant Staphylococcus aureus
PCR	polymerase chain reaction
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RCT	Randomized controlled trial
RR	Relative risk
TEP	Technical expert panel
VRE	Vancomycin resistant enterococci
WMD	Weighted mean differences