Comparative Effectiveness Review
Number 172

Early Diagnosis, Prevention, and Treatment of *Clostridium difficile*: Update

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Early Diagnosis, Prevention, and Treatment of *Clostridium difficile*: Update

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This report is based on research conducted by the Minnesota Evidence-based Practice Center (EPC) under contract to the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD (Contract No. 290-2012-00016-I). The findings and conclusions in this document are those of the authors, who are responsible for its contents; the findings and conclusions do not necessarily represent the views of AHRQ. Therefore, no statement in this report should be construed as an official position of AHRQ or of the U.S. Department of Health and Human Services.

None of the investigators have any affiliations or financial involvement that conflicts with the material presented in this report.

The information in this report is intended to help health care decisionmakers—patients and clinicians, health system leaders, and policymakers, among others—make well-informed decisions and thereby improve the quality of health care services. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information, i.e., in the context of available resources and circumstances presented by individual patients.

This report may periodically be assessed for the currency of conclusions. If an assessment is done, the resulting surveillance report describing the methodology and findings will be found on the Effective Health Care Program Web site at www.effectivehealthcare.ahrq.gov. Search on the title of the report.

Persons using assistive technology may not be able to fully access information in this report. For assistance contact EffectiveHealthCare@ahrq.hhs.gov.

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of systematic reviews to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. These reviews provide comprehensive, science-based information on common, costly medical conditions, and new health care technologies and strategies.

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 can help clarify whether assertions about the value of the intervention are based on strong evidence from clinical studies. For more information about AHRQ EPC systematic reviews, see www.effectivehealthcare.ahrq.gov/reference/purpose.cfm.

AHRQ expects that these systematic reviews will be helpful to health plans, providers, purchasers, government programs, and the health care system as a whole. Transparency and stakeholder input 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 email list to learn about new program products and opportunities for input.

We welcome comments on this systematic review. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 5600 Fishers Lane, Rockville, MD 20857, or by email to epc@ahrq.hhs.gov.

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Technical Expert Panel

In designing the study questions and methodology at the outset of this report, the EPC consulted several technical and content experts. Broad expertise and perspectives were sought. Divergent and conflicted opinions are common and perceived as healthy scientific discourse that results in a thoughtful, relevant systematic review. Therefore, in the end, study questions, design, methodologic approaches, and/or conclusions do not necessarily represent the views of individual technical and content experts.

Technical Experts must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

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Prior to publication of the final evidence report, EPCs sought input from independent Peer Reviewers without financial conflicts of interest. However, the conclusions and synthesis of the scientific literature presented in this report do not necessarily represent the views of individual reviewers.

Peer Reviewers must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals with potential nonfinancial conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential nonfinancial conflicts of interest identified.

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Early Diagnosis, Prevention, and Treatment of *Clostridium difficile*: Update

Structured Abstract

Objective. Update a 2011 review of differences in accuracy of diagnostic tests and the effects of interventions to prevent and treat *Clostridium difficile* infection (CDI) in adults.

Data sources. Medline®, the Cochrane Clinical Trials Registry, and Embase® from 2010 through April 2015 plus reference lists of included studies and recent systematic reviews.

Methods. Two investigators screened abstracts and full texts of identified references for eligibility. Eligible studies included studies of sensitivity and specificity for diagnostic tests in patients at risk for CDI. We included randomized controlled trials or high-quality cohort studies enrolling adult patients with CDI or suspected CDI for treatment interventions. Prevention studies also included adult patients at risk for CDI and observational study designs. Two investigators extracted data, assessed individual study risk of bias, and evaluated the strength of evidence for each comparison and outcome. Pooled estimates were analyzed to assess the efficacy and comparative effectiveness of a variety of treatments.

Results. We identified 37 diagnostic studies and 56 studies evaluating prevention or treatment interventions to update the review. High-strength evidence showed that nucleic amplification tests were sensitive and specific for CDI when using culture as the reference standard. Low-strength evidence was found that some institutional prevention interventions, such as antibiotic prescribing practices and transmission interruption (terminal room cleaning with hydrogen peroxide vapor and handwashing campaigns), reduce CDI incidence. Low-strength evidence also suggested that prevention programs can be sustained over several years. For CDI treatment, vancomycin is more effective than metronidazole (high-strength evidence), and the effect does not vary by severity (moderate-strength evidence). Fidaxomicin remains noninferior to vancomycin for the initial cure of CDI (moderate-strength evidence) but is superior to vancomycin for prevention of recurrent CDI (now high-strength evidence). Although both fecal microbiota transplantation (FMT) and probiotics were the subject of a significant number of new studies, the overall high risk of bias of many of these studies necessitated ratings of low strength of evidence. Specifically, low-strength evidence suggests that FMT may have a significant effect on reducing recurrent CDI. Similarly, low-strength evidence suggests that lactobaccilus strains and multiorganism probiotics also can reduce recurrent CDI. However, *Saccharomyces boulardii* was no more effective than placebo in preventing recurrent CDI. Evidence for FMT for refractory CDI was insufficient. Few studies reported adverse events; when reported, few events were noted.

Conclusions. Research on diagnostic testing for and interventions to treat CDI expanded considerably in 4 years. Nucleic acid amplification tests have high sensitivity and specificity for CDI. Vancomycin is more effective than metronidazole for initial CDI, while fidaxomicin is more effective than vancomycin for the prevention of recurrent CDI. FMT and lactobacillus probiotics to restore colonic biodiversity and improve patient resistance to CDI or recurrence have low-strength but relatively consistent positive evidence for efficacy.

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Introduction

Condition

Clostridium difficile infection (CDI) rates in the United States and the world have increased in the last decade, along with associated morbidity and mortality. *Clostridium difficile* is a gram-positive, anaerobic bacterium generally associated through ingestion. Various strains of the bacteria may produce disease generating toxins, TcdA and TcdB, as well as the lesser understood binary toxin. Our use of the term CDI indicates this review's focus is the presence of clinical disease rather than asymptomatic carriage of *C. difficile*. CDI symptoms can range from mild diarrhea to severe cases including pseudomembranous colitis and toxic megacolon and death.

Estimated U.S. health-care-associated CDI incidence in 2011 was 95.3 per 100,000, or about 293,000 cases nationally. Incidence is higher among females, whites, and persons 65 years of age or older.[1] About one-third to one-half of health-care onset CDI cases begin in long-term care, thus residents in these facilities are at high risk.[1,2] Incidence rates may increase by four- or five-fold during outbreaks.[3]

Community-associated CDI, where CDI occurs outside the institutional setting, is also on the rise, though still generally lower than institution-associated rates and may be in part due to increased surveillance. Estimated community-associated CDI was 51.9 per 100,000, or 159,700 cases in 2011.[1]. Community-associated CDI complicates measuring the effectiveness of prevention within an institutional setting.[3] Additionally, the pathogenesis of CDI is complex and not completely understood, and onset may occur as late as several months after hospitalization or antibiotic use.

The estimated mortality rate for health-care-associated CDI ranged from 2.4 to 8.9 deaths per 100,000 population in 2011.[1] For individuals ≥65 years of age, the mortality rate was 55.1 deaths per 100,000;[1] CDI was the 17th leading cause of death in this age group.[4]

Hypervirulent *C. difficile* strains have emerged since 2000. These affect a wider population that includes children, pregnant women, and other healthy adults, many of whom lack standard risk profiles such as previous hospitalization or antibiotic use.[5] The hypervirulent strains account for 51 percent of CDI, compared to only 17 percent of historical isolates.[6,7] Time from symptom development to septic shock may be reduced in the hypervirulent strains, making quick diagnosis and proactive treatment regimens critical for positive outcomes.

Diagnosis

Effective containment and treatment of CDI depends on accurate and swift diagnosis. An increasing number of diagnostic tests are designed to detect either the presence of the organism or toxins A and/or B with a variety of sensitivities, specificities, predictive values, biotechnologies used, training required, costs, and time-to-results. The testing strategies used in health systems are rapidly evolving. A study from 2008 showed that more than 90 percent of labs in the United States use enzyme immunoassay because it is fast, inexpensive, and easy to perform.[8] Just 3 years later, however, data showed that 43 percent of laboratories in the United States used nucleic acid amplification tests (NAAT) (e.g., polymerase chain reaction [PCR]).[9]

Clinically, CDI is diagnosed using tests such as: (1) immunoassays (including enzyme immunoassays, enzyme-linked immunosorbent assays, and immunochromatography assay), (2) tests for *C. difficile* toxins, and (3) amplification of *C. difficile* DNA, through means such as PCR and loop mediated isothermal amplification (LAMP). Some diagnostic testing strategies rely on

two-step procedures, the first being a sensitive, inexpensive, fast screen for the presence of the organism and, if that is positive, a second test for toxins. Toxigenic culture and cell cytotoxicity neutralization assay are no longer standard practice and are not universally available. However, given the rapid evolution of testing strategies, studies of diagnostic test performance often use toxigenic culture or cell cytotoxicity neutralization assay as the reference standard. Clinicians are not always well informed on the best diagnostic test to use, the operating characteristics of the tests used in their practice setting, or the relatively low likelihood of a false negative result (e.g., evidence suggests retesting with the same test is common practice, yet not recommended).

Treatment Strategies

Although there is not yet consensus on the definitions of mild, moderate, or severe CDI, treatment strategies do differ based on disease severity. Treatment for mild to moderate CDI is generally metronidazole, in part because of concerns that overuse of vancomycin may contribute to increasing pathogen resistance. Vancomycin is recommended for severe initial incident CDI.[10] However, both vancomycin and metronidazole have been implicated in leading to increased frequency of vancomycin-resistant enterococci.[11] In 2011, the FDA approved a new agent, fidaxomicin, for the treatment of CDI. A previous review found that while fidaxomicin was not superior for the initial cure of CDI, recurrence was less frequent with fidaxomicin than with vancomycin.[12] Measuring cure, however, can be challenging; no specific consensus exists regarding symptom resolution, clearance of the organism, or recurrence of CDI.

Treatment for relapsed or recurrent CDI is even more problematic. CDI recurs in 15 – 35 percent of patients with one previous episode and 33 – 65 percent of patients with more than two episodes.[13] Currently, clinicians choose from a number of antibiotics, dosing protocols, and adjunctive treatments (such as the use of antimicrobials, probiotics, toxin-binding agents, and immune-system enhancing agents).[14-16] The goal of most adjunctive treatments is to reduce patient susceptibility to relapse or reinfection. Fecal microbiota transplantation (FMT) in particular has garnered significant clinical interest. FMT transfers fecal microbiota from a healthy individual to a CDI patient to restore a healthy gut microbiota.

Prevention

Not all people who acquire *C. difficile* develop CDI; thus prevention measures can target reducing both the spread of the bacteria or spores and patient susceptibility to infection. One study statistically modeled CDI within the hospital setting and suggested that reducing patient susceptibility to infection is more effective in reducing CDI cases than lowering transmission rates.[17] The likelihood of developing CDI depends on a number of factors that allow colonization and toxin production, including failure of the immune defenses, use of antibiotics, particularly broad-spectrum or multiple antibiotics, and changes to the intestinal microbiota. Known risk factors for CDI include older age, comorbidities, and use of gastric acid suppressant medications.[13] Mortality is associated with age, white blood cell count, serum albumin, and serum creatinine.[18] Risk profiles for recurrent CDI are similar.[19] Recent prevention efforts have included antimicrobial stewardship and using environmental and infection control strategies, as well as seeking to improve the patient's immune defenses through healthy digestive function and gut flora and improved nutrition.[20]

Preventing transmission of *C. difficile* within institutional settings depends on staff compliance with national guidelines and standards[20] and locally determined hygiene protocols. Unfortunately, protocols for some targeted hospital-associated infections may not be effective

against *C. difficile*. For example, the availability of alcohol hand rubs improved physician compliance and reduced Methicillin-resistant staphylococcus aureus (MRSA) infections,[21] yet *C. difficile* produces spores that can withstand hostile environments and are resistant to alcohol hand rubs and other routine antiseptics. Spores may be best removed by handwashing. Other institutional prevention strategies may be required as *C. difficile* transmission knowledge develops. For example, one study isolated *C. difficile* spores and cultured the bacterium from air samples in a United Kingdom hospital 4 to 7 weeks after the last confirmed CDI case in the ward.[22]

Scope and Key Questions

Scope of the Review

In December 2011, the Agency for Healthcare Research and Quality (AHRQ) published the results of Comparative Effectiveness Review (CER) No. 3, Effectiveness of Early Diagnosis, Prevention, and Treatment of *Clostridium difficile* Infection, prepared by the Minnesota Evidence based Practice Center.[12] This CER examined the evidence on the sensitivity and specificity of *C. difficile* infection laboratory diagnostic tests, the effectiveness of prevention strategies, and the effectiveness and harms of antibiotic and adjuvant treatments for adults with CDI. The review was intended for a broad audience of clinical and policy decisionmakers. In January 2014, AHRQ published a surveillance report assessing whether an update of CER No. 3 was warranted. The report found new evidence for all Key Questions (KQs), suggesting the results were out of date.[23]

Several main findings were reported in CER No. 3. For diagnostic testing, direct comparisons of commercially available enzyme immunoassays for *C. difficile* toxins A and B found no major differences in sensitivity or specificity. Limited evidence suggested that tests for genes related to *C. difficile* toxins production may be more sensitive than immunoassays, but that specificities were inconsistent. Moderate-strength evidence in favor of antibiotic restriction policies for prevention was found. While no antimicrobial was clearly superior for the initial cure of CDI, as noted above, recurrence was less frequent with fidaxomicin than with vancomycin. Many potential new treatments were examined, and of these, fecal microbiota transplants for multiple recurrences appeared promising. However, with the numerous new publications identified in the surveillance report, an update of the review was merited.

This update systematically reviewed and assessed the evidence for diagnosis, prevention, and treatment of *C. difficile* using the original report and newly available evidence. We used essentially the same search strategy and review methodology, minimally updated to meet current review methods guidance. We made some minor modifications to the Key Questions in order to focus the update on current clinical concerns and due to the scarce literature base. Specifically, we deleted several subquestions regarding treatment effectiveness for subgroups. Since there has been some growth in the diagnostic testing literature, and diagnostic testing continues to be an area of decisional conflict, we also added a subquestion for testing strategy effects on final patient or health system outcomes.

Key Questions

KQ1: How do different methods for detection of toxigenic *C. difficile* to assist with diagnosis of CDI compare in their sensitivity, specificity, and predictive values?

a. How do they differ overall?
b. Do performance measures vary with sample characteristics?
c. Does testing strategy impact patient health or health system outcomes?

KQ2: 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?

KQ3: What are the comparative effectiveness and harms of different antibiotic treatments?

a. Does effectiveness vary by disease severity?

KQ4: What are the effectiveness and harms of other interventions?

a. How do they differ overall?
b. In patients with relapse/recurrent CDI?

PICOTS

Table 1 provides the PICOTS (population, intervention, comparisons, outcomes, timing, and settings) for the KQs. The analytic frameworks can be found in Appendix A.

Organization of the Report

This report presents the systematic review update in a summary fashion to focus the readers' attention on the main messages. The Methods section provides a brief overview of methods used. As is noted in that section, greater detail on methods can be found in the review protocol. The Results section provides a summary overview with key messages for each KQ. Detailed analyses for results are provided in the Appendixes. A table of contents for the Appendixes is provided at the beginning of the Appendix document. The report concludes with the Discussion section that summarizes how findings have changed from the original systematic review, on-going issues, research needs, and limitations of the review.

Table 1. Review PICOTS

PICOT	KQ1 Included	KQ1 Excluded	KQ2 Included	KQ2 Excluded	KQ3-4 Included	KQ3-4 Excluded
Population	• Adults with clinical signs consistent with CDI	• Pediatric patients alone • Patients not suspected to have CDI; healthy subjects • Patients already diagnosed with CDI	• Primary prevention: Adults at risk for CDI • Recurrence prevention: Adults with clinical signs consistent with CDI	• Pediatric patients	• Adults with clinical signs consistent with CDI • Adjunctive to prevent CDI: Adults at risk for CDI • Adjunctive to prevent recurrence: Adults with clinical signs consistent with CDI	• Pediatric, nonhuman, in vivo, or healthy volunteers
Intervention	• Diagnostic tests for toxin producing *C. difficile* • Immuoassays (enzyme immunoassays [EIA], enzyme-linked immunoassays [ELISA] immunochromato-graphy assays) • Tests for toxins • Two step strategies • DNA amplification (polymerase chain reaction [PCR], loop-mediated isothermal amplification [LAMP])	• Tests of stool culture alone • Tests to validate a technique in "known" or proven samples • Tests in which the reference standard is not applied to all samples • Tests examining cost characteristics • Tests not commercially available in the U.S. • Tests only typing *C. difficile* strains • Tests establishing proof of concept for new testing techniques (such as fecal calprotectin)	• Antibiotic stewardship, education, bundled preventive programs, prebiotics or probiotics used as preventive measures • Hospital inpatient environmental cleaning, monitoring, or surveillance • Environmental cleaning for long-term care facilities	• None	• Standard antibiotic treatments: ○ Metronidazole ○ Rifaxamin ○ Vancomycin ○ Fidaxomicin • Nonantibiotic adjunctive treatments: ○ Fecal transplant ○ Immunoglobulin ○ Pre/probiotics ○ Toxin binding agents ○ Rifampicin • Other new treatments available in the U.S.	• Treatments approved outside of the U.S. that are not available in the U.S.

PICOT	KQ1 Included	KQ1 Excluded	KQ2 Included	KQ2 Excluded	KQ3-4 Included	KQ3-4 Excluded
Comparator groups	• Reference Standard: cell cytotoxicity assay and/or toxigenic stool culture • Comparators: any includable diagnostic test listed above as intervention • For health system and patient outcomes: historical data comparators may be used	• In-house laboratory tests not commercially available	• Usual prevention practices for prevention strategies	• None	• Standard antibiotic treatments: active treatments such as metronidazole or vancomycin • Nonantibiotic adjunctive treatments: placebo, active controls, usual care.	• None
Outcomes	• Sensitivity • Specificity • Predictive values • Time-to-results • Patient outcomes • Health system outcomes (such as improved outcomes for patients or measured improvement for health systems with respect to cost of care, length of stay, or rates of CDI)	• None	• CDI incidence rates • CDI complication rates • CDI mortality rates • Harms, such as increase in organism resistance, hospital cleaning staff safety (bundled prevention programs), infection by introduced probiotics, isolation harms • Intermediate Outcomes: ○ Appropriate antibiotic use ○ Positive environmental cultures. ○ Days to resolution of symptoms (shorter window for transmission) • Other prevention strategy-related process variable demonstrating prevention strategy was taken up	• Studies that do not report CDI incidence rates and tie incidence to the inter-mediate process measures. For example, studies that only report environmental swabbing and culture for outcomes.	• Mortality • Recurrence (study author defined) • Clearance (study author defined) • Complications • CDI-related colectomy rate • Symptom resolution (study author defined) • Harms, such as delayed treatment response	• None
Timing	• Time to test results • For patient or health system outcomes: no	• None	• Variable	• None	• Variable, generally from 4 weeks to several months	• None

PICOT	KQ1 Included	KQ1 Excluded	KQ2 Included	KQ2 Excluded	KQ3-4 Included	KQ3-4 Excluded
	specific time requirement					
Setting	• Healthcare facilities: outpatient, inpatient, extended	• None	• Healthcare facilities: outpatient, inpatient, extended care	• None	• Healthcare facilities: outpatient, inpatient, extended care	• None

CDI = *Clostridium difficile* infection; EIA = enzyme immunoassay; ELISA = enzyme-linked immunoassay; KQ = Key Question; LAMP = loop mediated isothermal amplification; PICOTS = population, intervention, comparators, outcomes, timing, settings; PCR = polymerase chain reaction

Methods

The methods for this CER update follow the methods suggested in the AHRQ Methods Guide for Effectiveness and Comparative Effectiveness Reviews (available at www.effectivehealthcare.ahrq.gov); certain methods map to the PRISMA checklist.[24] All methods and analyses were determined *a priori*. We recruited a technical expert panel to provide high-level content and methodological expertise feedback on the review protocol. This section summarizes the methods used.

Literature Search Strategy

Our search methods were essentially the same as were used for CER No. 3. We searched Ovid MEDLINE, and Cochrane Central Register of Controlled Trials (CENTRAL) from 2011 to April 2015 to update CER No. 3. The keyword search for 'difficile' is highly specific yet sensitive to *C. difficile* related articles. The search algorithm is provided in Appendix B.

We conducted additional grey literature searching to identify relevant completed and ongoing studies. Relevant grey literature resources included trial registries and funded research databases. We searched ClinicalTrials.gov and the International Controlled Trials Registry Platform (ICTRP) for ongoing studies. Scientific information packet (SIP) letters and emails were sent to relevant industry stakeholders to request submission of published and unpublished information on their product(s). Grey literature search results were used to identify studies, outcomes, and analyses not reported in the published literature to assess publication and reporting bias and inform future research needs.

Studies were included in the review based on the PICOTS framework outlined in Table 1 and the study-specific inclusion criteria described in Table 2.

Table 2. Study inclusion criteria

Category	Criteria for Inclusion
Study Enrollment	Studies that enroll adults with suspected CDI
Study Design and Quality	Any: Systematic reviews with relevant questions of fair or good quality (see Risk of Bias section below); must include risk of bias assessment with validated tools Diagnosis: Studies of diagnostic accuracy assessing the operating characteristics of commercially available diagnostic test(s) for CDI in adult patients suspected of having CDI that include CCNA or toxigenic culture as the reference standard applied to all samples Prevention: RCTs, nonrandomized controlled trials, prospective cohort studies, retrospective cohort, time series, and before/after trials will be included. Cohort studies must include a comparator and appropriate methods to correct for selection bias. Due to larger available literature for antibiotic stewardship, before/after trials are excluded. Standard treatment: RCTs, nonrandomized controlled trials, and prospective cohort studies will be included for each population and treatment option. Prospective studies must include a comparator and appropriate methods to correct for selection bias. Studies specifically addressing treatment harms may also include retrospective and case series designs. Nonantibiotic standard treatment: RCTs, nonrandomized controlled trials, prospective cohort studies, and case series (at least 10 subjects) will be included for each population and treatment option. Prospective studies must include a comparator and appropriate methods to correct for selection bias. Studies specifically addressing treatment harms may also include retrospective and case series designs. For all KQs: Observational studies that do not adequately report study information to allow the abstraction of time sequences for treatment and followup duration or have indeterminable numerators and denominators for outcomes and adverse event rates

Category	Criteria for Inclusion
	were excluded at the abstraction phase.
Time of Publication	Update from previous systematic review. We scanned 2010 forward to assure all published literature was identified.
Publication Type	Published in peer reviewed journals
Language of Publication	English language publications will be included because that literature best represents interventions available in the United States. However, the search was not limited by language so that potential language bias could be assessed

CCNA = cell cytotoxicity neutralization assay; CDI = *Clostridium difficile* infection; RCT = randomized controlled trial

Study Selection and Data Extraction

We reviewed bibliographic database search results for studies relevant to our PICOTS framework and study-specific criteria. All studies identified at title and abstract as relevant by either of two independent investigator underwent full-text screening. Two investigators independently performed full-text screening to determine if inclusion criteria were met. Differences in screening decisions were resolved by consultation between investigators, and, if necessary, consultation with a third investigator. Appendix C provides a list of articles excluded at full text.

We first assessed the relevance of systematic reviews that met inclusion criteria. If we determined that certain Key Questions or comparisons addressed in the previous systematic review were relevant to our review, we assessed the quality of the methodology using modified AMSTAR criteria.[25] When prior systematic reviews were assessed as sufficient quality, and when the review assessed strength of evidence or provided sufficient information for it to be assessed, we used the conclusions from that review to replace the *de novo* process. If additional studies on these comparisons were identified, we updated the systematic review results. We then abstracted data from eligible trials and prospective cohort studies not included in previous systematic reviews that addressed comparisons not sufficiently addressed by a previous eligible systematic review. One investigator abstracted the relevant information directly into evidence tables. A second investigator reviewed evidence tables and verified them for accuracy.

Risk of Bias Assessment of Individual Studies

Risk of bias of eligible studies was assessed by two independent investigators using instruments specific to each study design. For diagnostic studies, we used the QUADAS-2 tool.[26] For randomized controlled trials (RCTs), questionnaires developed from the Cochrane Risk of Bias tool were used. We developed an instrument for assessing risk of bias for observational studies based on the RTI Observational Studies Risk of Bias and Precision Item Bank[27] (Appendix D). We selected items most relevant in assessing risk of bias for this topic, including participant selection, attrition, ascertainment, and appropriateness of analytic methods. Study power was assessed in 'other sources of bias' in studies with data that were not eligible for pooling. Overall summary risk of bias assessments for each study were classified as low, moderate, or high based upon the collective risk of bias inherent in each domain and confidence that the results were believable given the study's limitations. When the two investigators disagreed, a third party was consulted to reconcile the summary judgment.

Data Synthesis

Evidence and summary tables followed those used for CER No. 3 wherever possible. Information from individual studies reviewed in CER No. 3 were brought forward into this

updated report when meta-analysis was performed using such information. Otherwise, tables show studies identified for the update and text notes if and how overall results from CER No. 3 were amended.

Where possible, we used data from previous reviews combined with data abstracted from newly identified studies to create new datasets for analysis. We summarized included study characteristics and outcomes in evidence tables. We emphasized patient-centered outcomes in the evidence synthesis. We used statistical differences to assess efficacy and comparative effectiveness and calculate the minimum detectable difference that the data allowed (β=.8, α=.05).

For diagnostic studies we looked at the reference standards and base contrasts on the type of reference standard and respective operating characteristics.[28,29] We focused on the differences between test category/methodology sensitivities and specificities rather than on specific test sensitivities and specificities themselves. Categories were Immunoassays for Toxin A/B, glutamate dehydrogenase (GDH), PCR, LAMP, and test algorithms. We pooled one-step NAAT (PCR or LAMP) studies using random effects models; diagnostic test algorithm studies that include NAAT tests (likely PCR) were pooled with other test algorithms. Data were analyzed in OpenMetaAnalyst. We calculated sensitivity, specificity, receiver operating characteristic (ROC) curves, and negative and positive likelihood ratios.[30] We used random effect models to pool data when clinically appropriate.

For studies that used multiple reference standards, such as culture, toxigenic culture, and cell cytotoxicity neutralization assay (CCNA), we used toxigenic culture as the reference standard. If different reference standards were used for specific subgroups (such as study site) and none was used across all the samples, then we used the reference standard that was used in interpretation of the index test.

For treatment studies, if certain comparisons could be pooled, we conducted meta-analyses using a random effects model. Data were analyzed in Stata I/C version 12.1.We calculated risk ratios (RR) and absolute risk differences (RD) with the corresponding 95 percent CI for binary primary outcomes. Weighted mean differences (WMD) and/or standardized mean differences (SMD) with the corresponding 95 percent confidence intervals (CIs) were calculated for continuous outcomes. We assessed the clinical and methodological heterogeneity and variation in effect size to determine appropriateness of pooling data.[31] We assessed statistical heterogeneity with Cochran's Q test and measure magnitude with I^2 statistic.

Strength of Evidence for Major Comparisons and Outcomes

The overall strength of evidence for select outcomes within each comparison were evaluated based on four required domains: (1) study limitations (internal validity); (2) directness (single, direct link between intervention and outcome); (3) consistency (similarity of effect direction and size); and (4) precision (degree of certainty around an estimate).[32] A fifth domain, reporting bias, was assessed when strength of evidence based upon the first four domains was moderate or high.[32] Based on study design and conduct, risk of bias was rated as low, medium, or high. Consistency was rated as consistent, inconsistent, or unknown/not applicable (e.g., single study). Directness was rated as either direct or indirect. Precision was rated as precise or imprecise. Other factors that may be considered in assessing strength of evidence include dose-response relationship, the presence of confounders, and strength of association. Based on these factors, the overall evidence for each outcome was rated as:[32]

- **High:** Very confident that estimate of effect lies close to true effect. Few or no deficiencies in body of evidence, findings believed to be stable.
- **Moderate:** Moderately confident that estimate of effect lies close to true effect. Some deficiencies in body of evidence; findings likely to be stable, but some doubt.
- **Low:** Limited confidence that estimate of effect lies close to true effect; major or numerous deficiencies in body of evidence. Additional evidence necessary before concluding that findings are stable or that estimate of effect is close to true effect.
- **Insufficient:** No evidence, unable to estimate an effect, or no confidence in estimate of effect. No evidence is available or the body of evidence precludes judgment.

Applicability

Applicability of studies was determined according to the PICOTS (population, intervention, comparator, outcome, timing, settings) framework. Study characteristics that may affect applicability include, but are not limited to, the population from which the study participants are enrolled, diagnostic assessment processes, narrow eligibility criteria, and patient and intervention characteristics different from those described by population studies of *C. difficile*.[33]

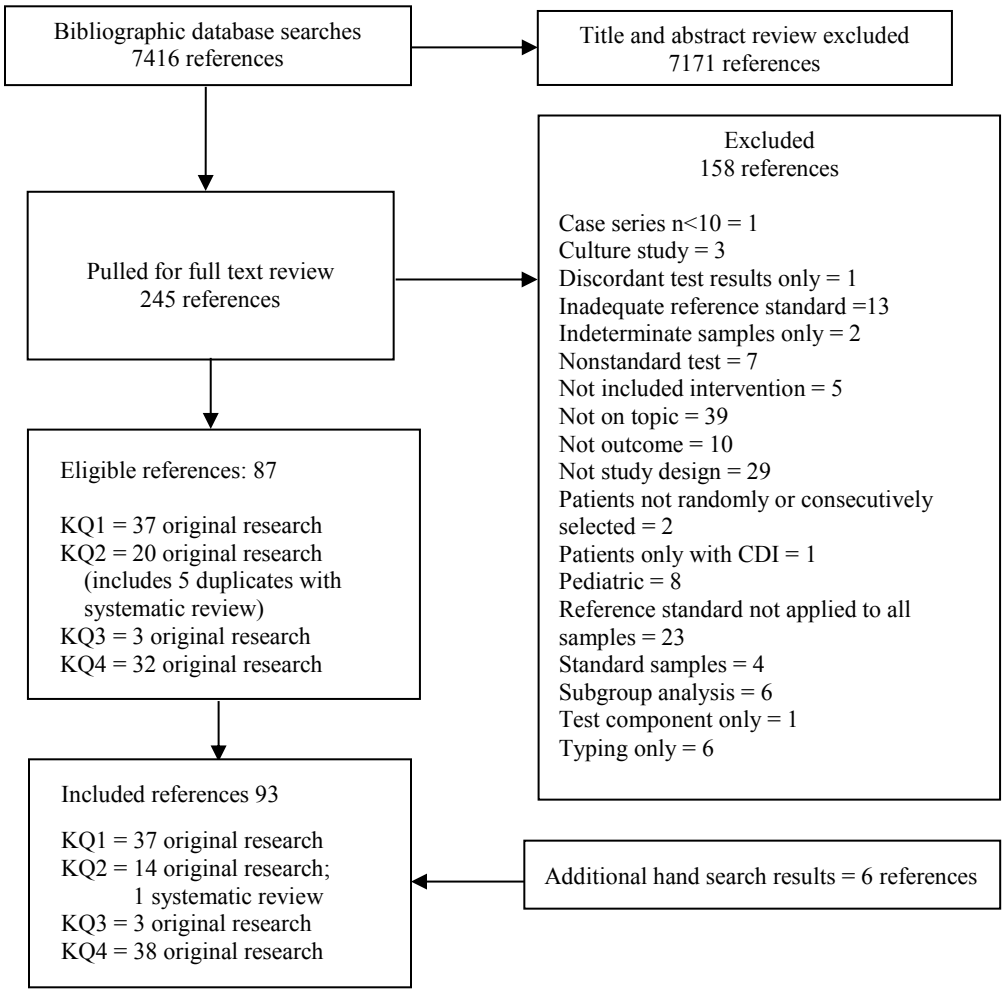
Applicability of studies of diagnostic accuracy of diagnostic tests for CDI may be influenced by the selection of patient samples in the studies included and the degree (if any) of delineation of the demographic and clinical characteristics of the studies' respective patient populations and how these characteristics compare with a local population. Further, certain diagnostic tests may not be available to all clinicians depending on local health system factors.

Results

Literature Search Results

We identified 7416 unique citations (Figure 1) from 2010 to April 2, 2015. After excluding articles at title and abstract, full texts of 252 articles were reviewed to determine final inclusion. Six articles were added through hand search.

Figure 1. Literature flow diagram



CDI = C. difficile infection; KQ = Key Question

The appendixes of this report provide detailed information about the included studies: evidence tables (Appendix E); risk of bias and quality assessments of original research and systematic reviews (Appendix F); detailed analyses (Appendix G); and detailed strength of evidence assessments (Appendix H).

KQ1: How do different methods for detection of toxigenic *C. difficile* to assist with diagnosis of CDI compare in their sensitivity, specificity, and predictive values?

Thirty-seven new studies evaluated diagnostic tests for CDI. Twenty-three studies were from Europe, six from the United States, three from Korea, two from Canada, and one each from Australia, Mexico, and Saudi Arabia. Twenty-six studies were performed at a single center and 11 studies were multicenter studies. (See Appendix C for evidence tables.) Most studies included only unformed stool specimens. Overall, these studies, when combined with the 13 studies from the original review, include data on eight named immunoassays for *Clostridium difficile* toxins A and B, four GDH tests, 11 test algorithms, one LAMP, and 10 PCR. The number of studies assessing the diagnostic accuracy of tests that detect genetic material from *Clostridium difficile* in feces (LAMP and PCR) increased considerably—19 studies in the update compared to only three in the original review.

Table 3 provides a summary of the findings.

Table 3. Summary of diagnostic test findings new with the update

Diagnostic Test	Study Information	Findings	Strength of Evidence
NAAT (LAMP and PCR) Tests	12 LAMP arms (1 test type), 31 PCR arms (10 test)	Sensitive (LAMP 0.95, CI 0.90 - 0.97; PCR 0.95, CI 0.93 - 0.96) and specific (LAMP 0.98, CI 0.96 -0.99; PCR 0.97, CI 0.96-0.98) for CDI	High (low study limitation, consistent, precise)
Tests for Toxin A/B	58 arms (8 test types)	Insensitive (0.70, CI 0.66 - 0.74) but specific (0.98, CI 0.97- 0.99) for CDI	Moderate (low study limitation, consistent, imprecise)
Tests for GDH	10 arms (4 test types)	Sensitive (0.90, CI 0.78 – 0.96) but less specific (0.94, CI 0.89 – 0.97) for CDI	Moderate (moderate study limitation, unknown consistency, precise)
Test Algorithms	11 arms (11 test types)	Insensitive (0.73, 0.62-0.82) but specific (1.00, 0.99-1.0) tests for CDI	Low (moderate study limitation, consistent, imprecise)

CDI = *Clostridium difficile* infection; CI = confidence interval; GDH = glutamate dehydrogenase; LAMP = loop-mediated isothermal amplification; NAAT = nucleic acid amplification tests; PCR = polymerase chain reaction

The general rankings provided in Table 3 are based on the overall pattern of results summarized in Table 4, which shows the sensitivity, specificity, and negative and positive likelihood ratios (forest plots and ROCs in Appendix G) comparisons (which are not derived from direct comparisons between test classes). In short:

- A negative LAMP assay is as effective at decreasing the probability that a patient has CDI as PCR and GDH assays and is more effective than Toxin A/B and algorithmic approaches. A positive LAMP assay is likely more effective at increasing the probability that a patient has CDI than PCR, Toxin A and/or B tests, and GDH assays but is less effective than algorithmic approaches.
- A negative PCR test is as effective at decreasing the probability that a patient has CDI as LAMP and GDH assays and more effective than Toxin A/B and algorithmic approaches. A positive PCR for CDI is more effective at increasing the post-test probability that a patient has CDI than a positive GDH test, similarly effective to LAMP and Toxin A/B assays, and less effective than algorithmic approaches.

- A negative immunoassay for Toxin A and/or B is as effective as algorithmic approaches but is less effective than PCR, LAMP, and GDH tests at decreasing the likelihood that a patient has CDI. A positive immunoassay for Toxin A and/or B is more effective at increasing the post-test probability that a patient has CDI than a positive GDH test, similarly effective to PCR, and less effective at increasing the probability that a patient has CDI than LAMP and algorithmic approaches.
- A negative GDH assay is as effective at decreasing the probability of CDI (albeit with less precision in the estimate) as PCR and LAMP and more effective than Toxin A and/or B tests and algorithmic approaches, but a positive GDH assay is less effective at increasing the probability that a patient has CDI than all the other test classes.
- A negative algorithmic test for CDI is the one of the least effective tests at decreasing the probability that a patient has CDI, while a positive test for CDI via an algorithmic test is the most effective approach to increase the post-test probability that a patient has CDI.

Heterogeneity within the classes is not easily explained by test type alone. The reasons for the differences in the operating characteristics between individual tests within the same class and between classes of tests are not well described in the studies, and while studies were selected to have good internal validity, studies may have differed significantly in the conduct of the tests and the patient populations.

We found no studies that met the inclusion criteria that provided sufficient sample characteristics to evaluate whether performance measures varied systematically based on health system, laboratory, training methods, or patient characteristics. Similarly, no studies that met the inclusion criteria evaluated the effect of different assays for CDI on health systems or patient outcomes.

Table 4. Summary of pooled diagnostic tests by test class

Test Characteristics	LAMP	PCR	Toxin A/B	GDH	Test Algorithms
Studies (k)	12	31	58	10	11
Sensitivity	0.95	0.95	0.70	0.90	0.73
95% CI	0.90-0.97	0.93-0.96	0.66-0.74	0.78-0.96	0.61-0.82
I^2 for heterogeneity	76.69	35.17	89.22	95.07	97.30
Specificity	0.98	0.97	0.98	0.95	0.995
95% CI	0.96-0.99	0.96-0.98	0.97-0.99	0.92-0.97	0.99-1
I^2 for heterogeneity	92.32	74.9	89.52	95.54	93.69
Positive Likelihood Ratio	50.79	29.81	32.41	17.65	130.83
95% CI	22.57-114.27	22.99-38.65	25.14-41.78	10.57-29.48	78.55-217.89
I^2 for heterogeneity	91.87	74.29	86.0	95.64	86.84
Negative Likelihood Ratio	0.04	0.035	0.22	0.08	0.22
95% CI	0.02-0.08	0.024-0.05	0.17-0.29	0.04-0.15	0.10-0.47
I^2 for heterogeneity	84.16	88.98	87.83	97.62	95.09

CI = confidence interval; GDH = glutamate dehydrogenase; LAMP = loop-mediated isothermal amplification; PCR = polymerase chain reaction

KQ2: What are effective prevention strategies?

Fourteen new articles examined prevention studies; one systematic review (which included five new studies), two on chlorhexidine gluconate bathing of patients, two on using hydrogen peroxide vapor for room disinfection, two on hand hygiene, one on disposable hydrogen peroxide wipes, one on gloving, and four on multicomponent interventions. None were controlled trials. Two used quasi-experimental designs, four (plus all relevant studies in the systematic review) used interrupted time series analysis, four used prospective pre/post designs

of single sites, and one used a retrospective pre/post designs for a single site. No study reported that it was conducted in an outbreak setting.

Overall, while study design and reporting improved somewhat from the original review, the evidence available to link prevention strategies to clinically important outcomes, such as CDI incidence, remains low strength. Table 5 provides a summary of the findings. (See Appendix G for evidence table.)

Table 5. Summary of prevention findings new with the update

Intervention	Study Information	Findings	Strength of Evidence
Antibiotic stewardship	1 systematic review (6 studies)	Appropriate prescribing practices associated with decreased CDI	Low (moderate to high study limitation, consistent, imprecise)
Bathing patients with chlorhexidine gluconate	2 studies		Insufficient (moderate to high study limitation, inconsistent, imprecise)
Hydrogen peroxide vapor with terminal room cleaning	3 studies (1 from original review)		Insufficient (high study limitation, consistent, imprecise)
Daily cleaning with hydrogen peroxide disposable wipes for high-touch surfaces	1 study		Insufficient (high study limitation, unknown consistency, imprecise)
Pulsed xenon ultraviolet light after terminal room cleaning	1 study		Insufficient (high study limitation, consistent, imprecise)
Handwashing campaigns	1 studies	Reduced CDI (rates fell from 16.75 to 9.49 cases per 10,000 bed days)	Low (moderate study limitation, unknown consistency, imprecise)
Multicomponent prevention interventions	15 studies (10 from original review)		Insufficient for effectiveness (high study limitation, consistent, imprecise)
	4 studies	Sustainable over several years	Low (moderate to high study limitation, consistent, imprecise)

CDI = *Clostridium difficile* infection; CI = confidence interval; RR = relative risk

Antibiotic Stewardship

One new high-quality systematic review[34] of antibiotic stewardship practices in inpatient settings included six studies (one RCT and five interrupted time series) and overlapped with the original review by one interrupted time series study.[35] The new systematic review categorized stewardship practices into audit and feedback, formulary restrictions and preauthorization interventions, guidelines implemented with feedback, guidelines without feedback, and computerized decision support programs. The six studies were evaluated as providing low strength of evidence that antibiotic stewardship programs reduced CDI incidence within the four antibiotic use program categories examined (audit and feedback, formulary restrictions, guidelines with feedback, and computerized decision support).[34] The review found no reports of harms associated with stewardship programs.

Transmission Interruption

Bathing patients was a new form of transmission interruption from the updated literature. Two moderate risk of bias studies found chlorhexidine gluconate bathing had inconsistent findings. One cluster randomized crossover study found no effect for daily bathing in five ICUs

in one medical center with either intention-to-treat or as-treated analysis.[36] This study did not assess compliance and experienced relatively low CDI rates in both study arms. In contrast, one quasi-experimental study found that bathing either 3 days per week or daily in three cohorts within one hospital reduced CDI rates.[37] While there were no concurrent controls, the cohort design allowed for some replicability and comparison. Changes in effects with dosing (daily versus three times per week) and the wash-out period strengthen the findings. Highest compliance rates were found in the ICU cohort versus general hospital or medical/surgical cohorts; however, regression models did not find compliance associated with CDI rates; the model estimated RR 0.71 (CI 0.57 – 0.89) three times per week for all cohorts.

Two new studies examined terminal room cleaning including hydrogen peroxide vapor.[38,39] Rooms known to have prior occupants with CDI or other disease-causing organisms are sealed and sporicidal hydrogen peroxide vapor is released into the rooms in a gassing process. Protocols are followed for sealing the vapors within the rooms until proper ventilation is complete. One pre/post study in the original review used hydrogen peroxide vapor as part of a multicomponent intervention to respond to an abrupt increase in nosocomial CDI infections (Table 4 in the original report). The decrease in CDI infections could not be separated from the natural decline that follows epidemics, nor could the effect of the vapor be separated from the multiple component intervention. Both new studies occurred in large (900-bed) hospitals not facing epidemic or hyperendemic events. One pre/post study of the vapor versus standard cleaning with bleach found a statistically significant reduction in CDI incidence.[38] In contrast, the quasi-experimental cohort study used hydrogen peroxide liquid in the standard cleaning solution and found a trend in reduction but no statistical difference in CDI.[39] Of the three studies, cleaning time ranged from 2 hours 20 minutes to 3 – 4 hours per room.

Hydrogen peroxide disposable wipes for daily cleaning of high-touch surfaces were used in one interrupted time series study.[40] The study reported CDI rates dropped from 54 to 39 cases/10,000 patients (p=.0005) when compliance was >80 percent. CDI rates were not different for any cleaning compliance level. The study did not report results compared to a control hospital, however, the control did not monitor compliance, and the patient population was younger.

One new pre/post study examined the effect of portable pulsed xenon ultraviolet light after terminal room cleaning on CDI incidence in a single 140-bed community hospital.[41] Rooms with a previous CDI patient were cleaned with a chlorine-based disinfectant product, followed by one 7-minute exposure in the bathroom and two 7-minute exposures in the main room to the ultraviolet light. The lights were also used in the operating suites at night, emergency departments in the early mornings, and other clinical areas as available. CDI incidence and hospital-associated CDI deaths and colectomies were found to decline.

Two new studies examined the effect of handwashing campaigns on CDI rates using uncontrolled interrupted time series design in 187 hospital trusts in the England[42] and 166 acute care hospitals in Ontario, Canada.[43] The original report did not locate studies that directly addressed the effect of handwashing on CDI. The two new studies examined campaigns that incorporated education and training programs and monitoring and feedback through either internal reports[42] or public reporting.[43] The program in England also empowered patients to remind healthcare workers of hand hygiene.[43] Based on the one moderate risk of bias study from England, low-strength evidence suggests that handwashing campaigns can reduce CDI incidence over a 3-year period, with rates falling from 16.75 to 9.49 cases per 10,000 bed days. The study also found via regression model that soap use (measured via centralized procurement of soap)

was independently associated with a slight reduction in CDI.[42] The other high risk of bias study found no statistical difference; however, the authors note having been unable to adjust for several possible confounders, including patient location in the hospital, type of hand product used, and concomitant introduction of other hospital-level infection prevention and control interventions.

One new pre/post study examined universal gloving with emollient-impregnated gloves.[44] This study does not add significantly to the original report's finding of low-strength evidence for gloving based on one RCT (Table 4 in the original report).

Cleaning and disinfection studies reported no adverse events noted for chlorhexidine gluconate bathing[37] or hydrogen peroxide vapor.[38,39] Changes in mortality associated with a stewardship can be considered a harm, if the difference in mortality is due to changes in prescribed antibiotics. The systematic review noted mortality as a primary outcome; of the six studies reporting CDI incidence, four reported mortality outcomes with no significant differences between comparisons. Otherwise, harms were not reported for antimicrobial stewardship programs.

Multiple Component Studies

Five new high risk of bias studies used multiple component interventions to address reducing CDI rates.[45-49] Three used pre/post designs[45,46,49] and two used uncontrolled interrupted time series approaches (both at single hospitals).[47,48] Ten studies with pre/post or time series with pre/post statistical approaches were identified for the original review (Table 4 in the original report).

Some differences in the literature from the original review are noted. First, the studies were framed as responding to general heightened concerns for CDI as a hospital associated pathogen rather than a localized epidemic or high endemic. Second, study followup was longer, ranging from 2 – 3 years for pre/post studies[45,46] to 27 – 81 months for time series studies.[47,48] Third, studies tended to include more information on CDI definitions and laboratory testing methods.

The study designs do not permit inferences for individual intervention components; however, the increase in study periods suggests that multiple component interventions can be sustained over several years.

KQ3: What are the comparative effectiveness and harms of different antibiotic treatments?

Three studies met inclusion criteria: an RCT comparing fidaxomicin to vancomycin,[50] a three-arm RCT comparing tolevamer (a toxin-binding resin) to metronidazole and vancomycin,[51] and a three-arm prospective cohort study comparing intravenous metronidazole to oral metronidazole and vancomycin.[52] Data from these new studies were combined with studies from the original report—a previous RCT of fidaxomicin versus vancomycin, and with three previous RCTs comparing metronidazole and vancomycin—to assess the efficacy of each drug.

Table 6 provides a summary of the findings. (See Appendix C for evidence tables.)

Table 6. Summary of standard treatment findings using pooled RCT data from original report and update

Intervention	Study Information	Findings	Strength of Evidence
Vancomycin vs. metronidazole	4 RCTs N=872	Initial Cure: favors vancomycin 83.9% vs. 75.7%; RR 1.08, 95% CI 1.02 – 1.15	High (moderate study limitation, consistent, precise)
	N=705	Recurrent CDI: not significantly different 16.5% vs. 18.7%; RR 0.89, 95% CI 0.65 – 1.23	Moderate (moderate study limitation, imprecise, consistent)
Fidaxomicin vs. vancomycin	2 RCTs N=1,111	Initial Cure: not significantly different 87.6% vs. 85.6%; RR 1.02, 95% CI 0.98-1.07	Moderate (low study limitation, consistent, imprecise)
	N=962	Recurrent CDI: favors fidaxomicin 14.1% vs. 26.1% RR 0.55, 95% CI 0.42-0.71	High (low study limitation, consistent, precise)
Any intervention: Treatment effect by disease severity	3 RCTs	Treatment results did not differ by disease severity	Low (moderate to high study limitation, inconsistent, imprecise)

CDI = *Clostridium difficile* infection; CI = confidence interval; RCT = randomized controlled trial; RR = relative risk

Benefits

The findings that vancomycin is more effective for initial cure of CDI in adults is new to this update because of improved precision. While the results for fidaxomicin versus vancomycin are consistent with the original review, the strength of the evidence improved.

An observational study (n = 205) comparing oral metronidazole, intravenous metronidazole, and vancomycin was also identified.[52] Results are similar to the RCTs, so this study was not included in the analyzed set. Initial cure was comparable for oral vancomycin (81 percent) and oral metronidazole (82.6 percent), but was significantly lower for intravenous metronidazole (52.4 percent; P <.001). Intravenous metronidazole performed significantly worse than either oral drug.

Time to resolution of diarrhea was reported in both the newly identified RCTs, with no differences observed based on treatment received. This outcome was not reported in the observational study. For both time to resolution of diarrhea and mortality, results did not differ from the original review's finding of no differences.

Harms

Only a slight change was observed based on the newly included studies. Similar to the original report, in the trial of metronidazole versus vancomycin, a similar percentage of subjects in each treatment arm experienced one or more serious adverse events. However, more subjects in the metronidazole group discontinued study medication because of an adverse event (11.2 percent versus. 6.5 percent; P = .06), whereas more subjects in the vancomycin group had evidence of nephrotoxicity (4.6 percent versus 1.0 percent, P = .02). Other harms, such as antimicrobial resistance, were not reported.

Disease Severity

In both new RCTs, pre-specified subgroup analyses among subjects with severe disease were performed to assess differences in outcome by treatment arm. Disease severity was generally

determined by one or more clinical values such as white blood cell counts, serum creatinine concentrations, body temperature, and severity of abdominal pain due to CDI. No significant differences were observed for initial cure for severity subgroups. Analyzing by disease severity did not change the overall study results. One study found less recurrence for vancomycin versus metronidazole for severe disease, but the results varied based on whether per-protocol, modified intention to treat, or strict intention to treat analyses were used. The observational study also looked for a treatment effect when stratified by disease severity and found no significant differences. The original review found insufficient evidence for treatment by severity based on one *post hoc* subgroup analysis for vancomycin versus metronidazole.

KQ4: What are the effectiveness and harms of other interventions?

Other treatments were categorized as (FMT, probiotics, or other. FMT was the largest updated literature set for nonantibiotic adjunctive therapy. Twenty-three new studies examined FMT for CDI: three RCTs and 20 observational studies, in addition to three observational studies carried forward from the original review. We identified 12 new studies on probiotic use: 10 RCTs and two observational studies, in addition to seven RCTs included in the prior report. We identified three new RCTs on other nonstandard therapies.

Table 7 summarizes the findings. (See Appendix C for evidence tables.)

Table 7. Summary of nonstandard treatment findings using data from original report and update

Intervention	Study Information	Findings	Strength of Evidence
FMT	3 RCTs, 23 case series N=751	Resolves diarrhea and prevents relapse in patients with recurrent CDI FMT given both for prevention of recurrence and for symptom resolution; often not clearly stated in studies.	Low (high study limitation, consistent, precise)
	3 contributing case series on refractory CDI N=19	Mixed findings on small number of patients	Insufficient (high study limitation, imprecise, unknown)
Lactobacillus vs. placebo	6 RCTs N=1251	Prevent CDI: favors lactobacillus RR 0.27, 95% CI 0.15-0.49	Low (moderate to high study limitation, consistent, imprecise)
S. boulardii vs. placebo	6 RCTs N=1244	Prevent CDI: not significant RR 0.77, 95% CI 0.38-1.54	Low (high study limitation, consistent, imprecise)
Multiorganism probiotics vs. placebo	5 RCT N=3960	Prevent CDI: favors multiorganism RR 0.50, 95%, CI 0.28-0.88	Low (high study limitation, consistent, imprecise)

CDI = *Clostridium difficile* infection; CI = confidence interval; FMT = fecal microbiota transplantation; RCTs = randomized controlled trials; RR = relative risk

FMT for Recurrent CDI

Twenty-three new studies addressed FMT for recurrent CDI; three were small size RCTs and the others were case series. Most studies were small, enrolling 12 to 94 individuals. Followup was variable, and ranged from 3 weeks to 8 years. In most cases, FMT was described as being administered after antimicrobials had reduced or resolved the acute symptoms of CDI, with the goal of limiting subsequent recurrence.

The three RCTs are noteworthy. One unblinded, three-arm RCT, conducted in the Netherlands, enrolled 43 adults with recurrent CDI (mean age 70, 43 percent women).[53] Patients were randomized to oral vancomycin, FMT, or vancomycin plus bowel lavage. Followup was 10 weeks and the endpoint was resolution of diarrhea. The study was stopped early due to a large difference between the FMT and comparator groups (81 percent versus 31 percent and 23 percent), largely due to an unexpectedly low response rate in the group randomized to vancomycin. FMT was administered via nasoduodenal tube. The resolution of diarrhea rate in the two vancomycin arms was considerably lower than the anticipated 60 percent. This may have been due to chance, and the 60-percent rate may have been achieved had the study treated the expected 38 patients per arm. However, without having run the full course, the study effect size remains uncertain.

Cammarota and colleagues conducted an additional unblinded trial of FMT via colonoscopy versus a vancomycin regimen that was given for at least 3 weeks, with the latter half given in a pulsed fashion (dosed every 2-3 days).[54] In patients with pseudomembranous colitis, the FMT protocol was amended after two patients to give FMT infusions every 3 days until resolution of colitis, versus the single infusion given to patients with CDI without pseudomembranous colitis. This study enrolled 39 subjects, with a mean age of 73. The primary endpoint was resolution of diarrhea associated with CDI at 10 weeks after the end of treatment. When analyzed by resolution after a single course of treatment (FMT or vancomycin), 65 percent of subjects had resolution of diarrhea with FMT, versus 26 percent with vancomycin. The authors noted that administering multiple courses of FMT increased the success rate to 90 percent in the FMT group, and that multiple antibiotic courses increased the success rate to 53 percent in the vancomycin group. This study was also stopped early after an interim analysis.

Youngster and colleagues conducted an unblinded RCT that randomized 20 individuals with recurrent CDI (mean age 54) to colonoscopic or nasogastric administration of FMT.[55] The study endpoint was resolution of diarrhea without relapse within 8 weeks. The authors found no difference between the two modalities of FMT administration, with an overall success rate of 70 percent after one treatment.

Based on a qualitative analysis of the unpooled data (Appendix G), low-strength evidence showed that FMT resolves diarrhea and prevents relapse in people with recurrent CDI.

FMT for Refractory CDI

Three studies reported outcomes for FMT in individuals with refractory CDI (defined as an episode that did not respond to antibiotic treatment; clearly identified by study authors). All were from case series, totaling 19 individuals.[56-58] Overall, there was insufficient strength of evidence supporting the role of FMT in refractory CDI. Unfortunately, few FMT studies provided detailed patient information to identify whether included patients could be considered refractory. For instance, in one study of 94 patients receiving FMT for recurrent or refractory CDI, there was not a detailed accounting of how many had refractory versus recurrent disease.[59]

Probiotics for CDI

Nineteen studies reported use of probiotics as adjunctive treatment for CDI: Ten RCTs and two observational studies were newly identified, while seven RCTs were included in the prior report. With 17 RCTs to provide a best evidence base, the observational studies will not be discussed further.

In all studies, probiotics were administered as an adjunct to standard antibiotic treatment to prevent CDI. All studies included adult inpatients or outpatients with a mean reported age of 50 to 77 years. The studies enrolled 40 to 2981 subjects. The probiotics tested were lactobacilli species in six studies, saccharomyces species (*S. boulardii*) in six studies, and multiorganism in five studies: both lactobacillus and saccharomyces species in one study, lactobacillus and bifidobacterium in two studies, a four-strain preparation of three lactobacilli and bifidobacterium in one study, and VSL#3 in one study.

For quantitative analysis, we categorized probiotics as single organism (lactobacillus organisms only), *S.boulardii*, or multiorganism (e.g., multistrain preparation of lactobacilli and bifidobacteria). Overall, we found low-strength evidence that probiotics containing only lactobacillus organisms are more effective than placebo in preventing an acute episode of CDI, predominantly driven by one moderate risk of bias study that also demonstrated dose response. We found low-strength evidence that probiotics containing *S.boulardii* given as adjunct to standard antimicrobial therapy are comparable to placebo in preventing an episode of CDI. We also found low-strength evidence that the multiorganisms are more effective than placebo.

Other Treatment Agents for CDI

Rifaximin versus placebo after standard antibiotic for CDI was examined by Garey and colleagues.[60] Rifaximin is a nonabsorbable antibiotic with FDA approval to treat traveler's diarrhea. Sixty-eight individuals with CDI (mean age 61, 50 percent were women) were treated for 20 days. After 3 months of followup, authors reported no statistically significant difference in recurrent CDI between groups. Recurrent diarrhea was reported less likely in the rifaximin group, but this included self-reported diarrhea episodes without confirmed *C. difficile* toxins.

Human recombinant lactoferrin versus placebo was examined by Laffan and colleagues.[61] Human recombinant lactoferrin from breast milk has both anti-inflammatory and antimicrobial properties. The study randomized 30 residents of a long-term care facility beginning a new course of antibiotic, either with CDI or without, to human recombinant lactoferrin or placebo for 8 weeks. Mean age was 62, 64 percent were women, and 32 percent were black. The study endpoint was CDI incidence rates at days 14, 42, and 56. CDI rate did not differ statistically between groups.

Cholestyramine, a toxin-binding substance, was used to prevent CDI in one case series of 46 Lyme disease inpatients receiving ceftriaxone.[62] Three patients subsequently developed CDI. Patients received 4 g/day administered orally up to 1 hour after the intravenous ceftriaxone but more than 1 hour before the evening meal. Patients were followed 30 days after treatment end. The lab-confirmed CDI rate of 6.5 percent (3/46 patients) was lower than reported rates for other patients receiving cefrtriaxone.

Harms of Adjunctive Treatments

Harms for FMT were available in the updated literature set. Adverse events after FMT in the single small RCT were diarrhea, cramps, belching and nausea, and constipation.[53] Serious adverse events included one hospitalization and two cases of infections, unrelated to FMT. Risk of infection from FMT appears to be low, but is also dependent on the donor screening and testing process, especially for pathogens without widely available diagnostic tests, such as norovirus or rotavirus. Upper gastrointestinal bleeding was reported in one study with nasogastric administration of FMT.[63] Other serious adverse events were peritonitis, pneumonia, and microperforation of the colon.[64] All-cause mortality after FMT ranged from 0 – 25 percent

when reported, depending on the length of followup. Mortality rates after FMT were higher in individuals with refractory compared with recurrent CDI. However, variable followup time, differences in baseline comorbidities, and especially the lack of any control group make placing this figure into context difficult. Whether deaths were due to FMT or reflected the overall poor health status of individuals undergoing FMT was unclear, particularly for those with refractory CDI. While one study reported a followup interval of up to 8 years,[65] the followup for the majority of studies was 3 months or less. Therefore, the long-term (greater than 3 months) adverse effects of FMT are largely unknown.

Sixteen of 19 studies of probiotics as adjunctive treatment for CDI reported data on adverse events (see Appendix Table E5). Treatment with probiotics was not associated with increased risk of adverse events in any of the studies. No serious adverse events were reported that were attributed to probiotic treatment, although followup was typically 4 weeks or less, with two RCTs extending followup to 12 weeks. Given the importance of the potential harm due to probiotics, we reiterate from the original report that fungemia may be a serious potential harm associated with administration of probiotics for CDI in critically ill patients.[66]

Discussion

Overview

This update identified a few notable changes from the original review to support the diagnostic, preventive, and treatment practices for CDI. Table 8 provides a summary of the findings presented in this update along with the findings of the original report.

Table 8. Summary of findings for update and original review

Key Questions	Level of Evidence, Update	Level of Evidence, Original Report	Summary/Conclusion/Comments
KQ1 - Diagnostics			
Nucleic acid amplification tests	High level	NA	Sensitive and specific for CDI
Enzyme tests for toxins A/B	Moderate level	NA	Sensitive but less specific for CDI
Assay tests for glutamate dehydrogenase	Moderate level	NA	Specific but less sensitive for CDI
Test Algorithms	Low level	NA	Multi-step tests specific but less sensitive for CDI
KQ2 - Prevention			
Antibiotic use	Low level	Low level	Appropriate prescribing practices associated with decreased CDI
Gloves	Low level	Low level	Use of gloves in hospital settings reduced CDI incidence
Disposable thermometer	NA	Low level	Use of disposable thermometers in hospital settings reduced CDI incidence
Bathing patients, chlorhexidine gluconate	Low level	NA	Bathing patients with chlorhexidine gluconate in hospital settings is insufficient
Handwashing/ alcohol gel	Low level NA	Insufficient level Low level	Handwashing campaigns in hospital settings reduce CDI incidence No significant differences in CDI incidence for alcohol gel to reduce MRSA transmission.
Disinfection	NA Insufficient level Insufficient level Insufficient level	Low level Insufficient level Insufficient level NA	Intensive disinfection with chemical compounds (hypochlorite, aldehydes, hydrogen peroxide) that kill *C. difficile* spores for terminal room cleaning reduced CDI incidence Daily cleaning with hydrogen peroxide disposable wipes for high-touch surfaces is insufficient Hydrogen peroxide vapor treatment with terminal room cleaning evidence remains insufficient Pulsed xenon ultraviolet light after terminal room cleaning evidence is insufficient
Multiple component strategies	Insufficient level	Insufficient level	Body remains insufficient to draw conclusions.
Sustainability	Low level	Insufficient level	Longer-term studies of prevention program roll-outs suggest programs are sustainable

Key Questions	Level of Evidence Update	Level of Evidence Original Report	Summary/Conclusion/Comments
KQ3 – Standard Treatment			
Vancomycin versus Metronidazole	High level Moderate level	Moderate level Low level	Vancomycin more effective in achieving initial cure No difference between groups for recurrent CDI
Fidaxomicin versus Vancomycin	Moderate level High level	Moderate level Moderate level	No significant differences in initial cure. Decreased recurrence among those receiving fidaxomicin
Effect by disease severity	Low level	Insufficient level	Reported results by treatment arm are present regardless of severity
All other comparisons of standard treatments	NA	Low level for all comparisons	Vancomycin versus bacitracin, vancomycin versus nitazoxanide, vancomycin high versus low dose, metronidazole versus nitazoxanide, and metronidazole versus metronidazole plus rifampin. No differences
Strain of organism	NA	Low level	One RCT (fidaxomicin versus vancomycin) demonstrated decreased recurrence among those receiving fidaxomicin when the infecting organism was a non-NAP1 strain
Patient characteristics	NA	Insufficient level	No comparative data were available
Resistance of other pathogens	NA	Insufficient level	No data were available
KQ4 – Other Treatment			
Treating CDI, active control	NA	Low level	Probiotics, prebiotics, *C. difficile* immune whey, and colestipol, are not more effective in treating CDI than standard antibiotic treatment with oral vancomycin or metronidazole
Treating CDI, placebo	NA	Low level	Administration of a probiotic with live bacteria to treat CDI in critically ill patients increases risk for greater morbidity and mortality from fungemia without any known benefit
Treating recurrent CDI	Low level Insufficient level	Low level NA	Fecal microbiota treatment is effective in treating recurrent CDI Data insufficient for patients with refractory CDI
Preventing CDI	NA	Low level	Prebiotics and monoclonal antibodies are not more effective than placebo for primary prevention of CDI
Preventing recurrent CDI	Low level Low level NA	Low level Low level Moderate level	Probiotics using lactobacillus or multiorganism strains are more effective than placebo for reducing recurrent CDI Probiotics using *S. boulardii* are not more effective than placebo for reducing recurrent CDI Monoclonal antibodies are effective in preventing recurrence of CDI

CDI = *Clostridium difficile* infection; NA = not applicable

KQ1—Diagnostic Tests

The literature has shown a strong shift from immunoassays to nucleic acid amplification tests, mirroring the evolution of clinical practice for diagnosis of CDI. Given the greatly

increased published literature of diagnostic studies, we were disappointed at the lack of eligible studies of the impact of diagnostic tests on patient or health system outcomes; that is, does more accurate and expeditious diagnosis of CDI lead to improved outcomes for patients or measured improvement for health systems (with respect to cost of care, length of stay, or rates of CDI). Although some retrospective studies described changes in incidence and prevalence in an institution before and after implementation of a new testing strategy, these generally did not include verification of the reported incidence and prevalence with an acceptable reference standard and are thus difficult to interpret. One study that did not meet our inclusion criteria (due to an inadequate reference standard) showed that a health system's change from a testing strategy based on culture and cytotoxicity assay to PCR or algorithmic approaches decreased time to results, decreased vancomycin and metronidazole use in patients without CDI, and decreased time to appropriate therapy in patients with CDI.[67]

Interpreting the findings of the diagnostic testing evidence requires a nuanced approach. First, the reference standard used to define the presence or absence of disease and the implications of that reference standard must be considered. We opted to use toxigenic culture or CCNA performed on loose stool as the reference standard; this is not a clinical reference standard that includes clinical information such as severity of disease and antibiotic exposure.

Further, the pretest probability of CDI (and the severity of the CDI, if present) varies with patient characteristics as well as clinical setting, with inpatient populations having higher prevalence and severity of disease. Determining the presence or absence of a given disease is not simply the obverse and reverse of each other, respectively. In the inpatient setting—from which the vast majority of patients in the studies included in this report were drawn—a clinician's priority is on ruling out disease (and not treating for CDI), and thus a higher false positive rate is likely acceptable. Since specificity was uniformly quite good across test classes, the difference in performance between classes of tests for CDI appears to be derived mostly from differences in sensitivity between classes. This has significant implications for testing strategy selection, since ensuring a negative test is accurately ruling out disease is likely of more clinical importance than ensuring that a positive test (in a patient with signs and symptoms consistent with CDI) represents true CDI. Thus, we believe the differences in sensitivity and negative likelihood ratios to have clinical importance.

There was substantial heterogeneity in the studies of diagnostic accuracy from both measured and unmeasured (and thus undescribed) sources. The prevalence of CDI in the study population is one of the few consistently published population characteristics. The prevalence of CDI in the examined studies varied widely, between 6 and 48 percent. While sensitivity and specificity should theoretically not vary with prevalence, they often do vary in studies of diagnostic testing, and future work should examine how the prevalence of CDI (likely a reflection of the population in a study and local testing behavior) influences the measured operating characteristics. There are many other undescribed clinical variables that may differ between populations and lead to heterogeneity. For example, training and implementation procedures generally were not described in detail. Lastly, the reference standard for each study was performed according to local protocols and, while used as the definition for the presence or absence of disease in the studies, may vary substantially, leading to different prevalence and operating characteristics. Unless a large, prospective trial is performed in which all reference standards (toxigenic culture and/or CCNA) are performed centrally to avoid local variation, these limitations are unlikely to be avoided in future studies.

The diagnostic studies included in this report included patients only with suspected CDI and thus the operating characteristics (that is, sensitivity and specificity) are defined in patients with suspected CDI, not general patients with diarrhea or healthy patients. Thus, these tests should only be interpreted in patients similar to patients enrolled in the included studies and clinicians must be aware of the prevalence of CDI in their own local population in determining whether they choose to employ a more sensitive or specific testing strategy. Further, the reference standard used (toxigenic culture or CCNA) does not include clinical information.

For this update we used a different approach to examine diagnostic tests, pooling studies by test class, since the selection of tests from within a test class for use at a certain institution will likely depend on both the operating characteristics of the test class and individual test as well other factors including cost and vendor preference. We found moderate to high evidence that NAAT tests (one LAMP, 10 PCR) are highly sensitive and specific. All other diagnostic tests (eight Toxin A/B immunoassays, four GDH immunoassays, and 11 test algorithms) were high in sensitivity and/or specificity but when compared with NAAT tests, lack the same combination of high sensitivity and specificity.

Test algorithms, intended to make the best of individual test strengths performed in series, did not perform as a class as well as NAAT tests. Clinical interest in test strategies has declined because of the issue of what to do when a positive initial test is followed by a negative test. Many clinicians will continue treating based on the first test because of the uncertainty (increased probability of CDI after the test), and because the test is usually ordered based on the clinician's pretest assessment of the patient's probability of CDI. Dichotomous, positive/negative results are easier for clinicians to interpret and require less laboratory followup.

NAAT tests come with a different set of concerns, including whether switching to NAAT will falsely inflate nosocomial CDI rates; these highly sensitive tests may identify people who are asymptomatic carriers or patients with diarrhea from a cause other than CDI. Since NAAT tests nearly approximate toxigenic culture in sensitivity and specificity, implementation of a NAAT-based testing strategy may lead to a higher observed prevalence/incidence compared with other testing strategies. Further research is required to determine if NAAT-based testing strategies lead to overtreatment for CDI in patients who are asymptomatic carriers or have diarrhea from another cause. It is likely that NAATs will be used as the reference standard in future studies and the implications of this change must be considered in interpretation of these studies.

To assume that one "best" test exists for all healthcare purposes is an oversimplification, especially with respect to populations with different pretest probabilities, as previously discussed. The need to understand the pretest probability of CDI extends to the stool samples for which laboratories will perform testing for *C. difficile* and ensuring that only unformed specimens from patients at risk for CDI are tested to avoid false positive tests that lack clinical significance and may lead to overtreatment. In addition, optimal testing strategies between health systems may differ on factors other than test analytics, including start-up costs, expertise, incremental (per-assay) cost, and other factors. Further, clear delineation must be made between the most effective test characteristics for at-risk individuals who may benefit from CDI treatment versus for population surveillance or epidemiologic evaluation.

KQ2—Prevention

The prevention literature remained generally low-strength, and little evidence connects prevention strategies directly to patient-related outcomes such as CDI incidence. Studies of

transmission interruption techniques were often excluded due to lack of patient-related outcomes (they used swabbing and culturing to assess the presence of *C. difficile* organisms or spores). However, we did identify some small updates to the original review. Low-strength evidence supports handwashing campaigns. Low-strength evidence also suggests that prevention programs are sustainable in the long-term. However, it remains difficult from a research perspective to definitively state that bundled, multicomponent interventions are effective, as each remains relatively unique to the specific location and the components included in that bundle. The information is still insufficient to answer which components are essential or what might be added.

Low-strength evidence continues to support antibiotic prescribing practices. Again, none of the studies explicitly addressed potential harms of changes in antibiotic use policy, such as the possibility that preferred drugs will be less effective than the drugs physicians are discouraged from using, or that preferred antimicrobials might have greater costs or greater toxicities unrelated to CDI.

KQ3—Standard Treatment

Three new studies of standard treatment raised confidence in several findings from the original review. We increased strength of evidence from moderate to high for vancomycin as a more effective agent than metronidazole for CDI, with moderate-strength evidence of the effect regardless of severity. Current treatment guidelines from the Infectious Diseases Society of America (IDSA) support vancomycin as the drug of choice for severe CDI, and metronidazole as the drug of choice for mild to moderate CDI.[68] This review's finding is consistent with reconsidering the preferred agent for mild to moderate CDI, although the long-term effects of increased vancomycin use are unknown. This is especially true in light of scant evidence to suggest that vancomycin promotes the emergence of vancomycin-resistant enterococci more so than other agents and a decrease in the price differential between metronidazole and vancomycin.

A second important finding is continuing moderate-strength evidence that fidaxomicin is similar to vancomycin for the initial cure of CDI, and increased strength of evidence for fidaxomicin is superior for the prevention of recurrent CDI. Since the desired outcome with CDI treatment is cure of the initial illness without subsequent recurrence, this finding ought to prompt consideration of fidaxomicin for the initial treatment of CDI. This is especially relevant to the treatment of CDI since each episode of recurrence increases the likelihood of further episodes. Since fidaxomicin was licensed after publication of the most recent IDSA guidelines, they include no mention of fidaxomicin. Accordingly, its role in treating CDI has been a topic of considerable discussion. A recent cost-benefit analysis concluded that the per-course price of fidaxomicin would need to decrease by more than 10-fold in order to make such use cost-effective.[69] The current high cost of fidaxomicin prompted its manufacturer to seek and obtain a new technology add-on payment from the Centers for Medicare and Medicaid Services. This add-on provides hospitals additional payment to offset fidaxomicin's high cost. Future guidelines will hopefully give clinicians guidance as to how to best use this agent to maximize the value seen in terms of reduced episodes of recurrent CDI.

A final updated finding is that in the observational study of intravenous metronidazole verses oral metronidazole and vancomycin, intravenous metronidazole performed significantly worse than either oral drug. This finding should be interpreted with caution given the observational nature of the study and the significant possibility of confounding. Since this finding largely

confirms current clinical practice, it will not likely have a major impact on the treatment of patients with CDI.

The findings in this review remain applicable for the general adult CDI patient population. Given the paucity of the literature, we were unable to assess findings for important subgroups of interest.

KQ4—Other Treatments

Adjunctive treatments in the updated literature have largely focused on restoring the colonic microbiome for the prevention of subsequent CDI, although a few explored different mechanisms such as toxin-binding (tolevamer, cholestyramine) and direct antimicrobial properties (lactoferrin). The diverse bacterial species residing in the human gut, commonly referred to as the colonic micobiome, provide host resistance to infection by *C. difficile*. Several factors, such as antimicrobial use and chemotherapy, disrupt the diversity of the colonic microbiome and lower the resistance to CDI. Antimicrobials are effective in treating CDI, but also disrupt the colonic biodiversity and do not address the necessary repopulation of these organisms. These changes make the host susceptible to recurrent episodes of CDI. Probiotics aim to recolonize the intestinal flora with nonpathogenic bacteria, while FMT involves the transfer of the entire microbiome from one individual or a pool of donors to the host.

Low-strength evidence supports FMT as a promising therapy for recurrent CDI. Our findings are consistent with another recent systematic review which provided greater detail regarding method and route of FMT, as well as donor characteristics, but did not include six recent studies included in this report.[70] Since our original review, numerous studies have addressed FMT for the treatment of recurrent CDI, including two small unblinded RCTs comparing FMT to vancomycin-based control groups, one small RCT comparing two different modalities of administration for FMT, and numerous case series. The case series ranged from small to medium size and provide a cumulative experience with FMT of 751 individuals, with reported success rates from 48 – 100 percent. However, the high probability of publication bias and the lack of control groups are major limitations. The data from the RCTs comparing FMT to vancomycin are encouraging, demonstrating a significant benefit for FMT, although the study risk of bias is high. Specifically, participants and providers were unblinded, both trials were stopped early, and the control groups in both of the trials had success rates from 23 to 31 percent, far lower than the 55-60 percent rates expected based on the sample size calculations published in the study protocols. Additionally, followup was limited in most studies; thus, the long-term consequences of FMT treatment are unknown.

Insufficient evidence exists for FMT for refractory CDI. In contrast to FMT for recurrent CDI—which is administered after a course of antimicrobial therapy has eliminated or greatly reduced symptoms of CDI, and whose main aim is to prevent subsequent recurrences—FMT for refractory CDI is administered to patients with ongoing symptoms of CDI despite antimicrobial therapy. Since the great majority of patients with CDI respond to initial antimicrobial treatment, studies of refractory CDI are inherently difficult.

The scientific and regulatory issues for FMT pose unique challenges, as there are no standard formulations, methods of quantifying, or assessing safety of stool. The composition of stool, and which constituents may be active in reducing recurrent CDI, is also currently unknown. Initial FDA guidance required an Investigational New Drug (IND) for any use of FMT. After significant public input, current FDA policy is to exercise enforcement discretion regarding IND requirements for FMT in specific situations. To proceed under enforcement discretion, FMT

must be used to treat CDI not responding to standard therapies, and the treating physician must obtain an informed consent including, at a minimum, discussion of both the investigational nature of FMT and its potential risks (www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm361379.htm). This guidance is subject to change as more evidence and experience accumulates on FMT, including the optimal route of FMT delivery. The sources of material for FMT are also variable and include use of unrelated or related donors. No standard criteria exist for screening donors, but several guideline documents have been developed. The optimal team to administer and oversee an FMT program is uncertain but may include gastroenterology, infectious diseases, pharmacy, infection control, nursing, and facility management.

The low-strength evidence supporting probiotics for the prevention of CDI is mixed. Preparations containing *S. boulardii* alone did not seem to significantly affect subsequent rates of CDI, whereas preparations containing lactobacillus strains or multiorganism mixes did significantly reduce rates of CDI. Notably, the studies aimed to examine probiotics for primary prevention of CDI among patients without a prior episode of CDI. Whether the findings apply to patients with a history of CDI (that is, to prevent recurrence of CDI) is unknown. Our findings are generally consistent with another systematic review, although we differed on the *S. boulardii* finding due to our review including one additional study.[71] Administering a course of probiotics to every patient taking antimicrobials would also be a rather substantial change in medical practice. The cost/benefit ratio of such a policy is unclear, based on the mixed and low-strength findings of this report, as is whether benefits could be conferred by ingesting probiotics in the form of yogurt, kefir, and other similar foods. Given the multitude of such foods available to consumers, the prospect of obtaining rigorous data on each seems unlikely.

Finally, rifaximin and lactoferrin were studied in separate small placebo controlled trials and oral cholestyramine in a case series of 46 patients regarding their ability to prevent subsequent episodes of CDI. Rifaximin was given after a course of standard antimicrobial therapy for CDI in hopes of preventing recurrent CDI. In contrast, lactoferrin and cholestyramine were given concomitantly to antibiotics prescribed for non-CDI indications in the hopes of preventing an initial episode of CDI. In the controlled trials the investigational agent reduced the incidence of subsequent diarrhea, but not confirmed CDI. CDI was confirmed in the cholestyramine study.

The bulk of the new studies of adjunctive treatment for the prevention of subsequent CDI involve efforts to reconstitute the colonic microbiome with either FMT or probiotics. The supporting evidence is low-strength. The FMT studies show a large treatment effect but are limited by methodological weaknesses. In contrast, the studies supporting probiotics demonstrate a less-impressive treatment effect. The FMT studies included patients with at least a single prior episode of CDI and, in many cases, multiple prior episodes. The probiotic trials, on the other hand, are examining primary prevention of CDI. Both primary and secondary prevention of CDI are important, particularly since the burden of CDI has significantly increased over the past 15 years.

Research Gaps

For diagnostic studies, in spite of the increased applicable evidence in this update, many differences persisted in each laboratories 'CDI reference standards and thus likely the standards' sensitivity and specificity. (There is not one standard toxigenic culture assay, for example, used in all laboratories.) Reference standards are used to determine CDI prevalence, but no reference

standard has perfect sensitivity and specificity. Even small differences in prevalence in a population may lead to markedly different predictive values.

The marked heterogeneity in the operating characteristics of the tests analyzed was puzzling. No tests have perfect operating characteristics; thus, we could not determine the clinical significance of the differences in operating characteristics between individual tests and classes of tests. Future studies should determine whether the differences in operating characteristics for the same proprietary test between laboratories are the result of patient/sample characteristics, prevalence of disease, test performance, reference standard performance, or other factors. The findings of these studies would likely be of significant pragmatic importance as new testing strategies are applied in health systems across the country. Best practices for testing that are independent of the manufacturer should be developed.

The criteria for future studies outlined in the previous report with a few modifications (in italics) should be applied to future multicenter studies: (1) use the most clinically relevant reference test performed in a centralized and/or standard fashion; (2) use explicit clinical criteria to select patients and stool specimens to be tested; (3) randomly assign patients to different diagnostic tests *(or perform and interpret multiple tests independently)*; and (4) use key clinical outcomes as study endpoints are needed. Also, studies should prospectively determine how inconclusive results will be handled, and all samples should be included in the determination of operating characteristics and the result of each test (if tests are applied serially) made available for analysis.

For prevention, the main obstacle to research continues to be the contextual setting. To design and conduct studies with adequate comparators to allow for causal inference is certainly challenging. Nonetheless, the field would benefit from such work. Indeed, study designs in this review update did improve, using pragmatic cluster trials and prospective data collection with interrupted time series. Further use of implementation science techniques may move the field forward. Additional studies of transmission interruption that follow results past culturing room swabs to clinical outcomes such as CDI incidence would also be of benefit. Given the disease burden in long-term care settings, studies examining interventions in these settings would also be welcome.

Future research needs for the treatment of the first episode of CDI include studies to identify subgroups of patients who derive the most benefit from fidaxomicin, including whether ribotype matters, and studies of new agents to further decrease the recurrence rate from the 14 percent observed with use of fidaxomicin. A few new agents are currently under investigation. (See Appendix Table I1.) Recurrent CDI is difficult for both patients and clinicians to manage; thus, lowering the recurrence rate as much as possible is a high clinical priority. Finally, since both the largest RCT of metronidazole verses vancomycin and pooled data from all such trials indicate that vancomycin is superior to metronidazole for the initial cure of CDI, further studies comparing these agents are not likely to be clinically useful.

Adjunctive treatments for CDI need more research. FMT is particularly challenging to research. It involves highly complex microbial mixtures that vary from donor to donor. Additionally, several delivery routes are used, including instillation of donor feces into the upper gastrointestinal (GI) tract via nasogastric or naso-jejunal tubes or in an oral capsule, instillation of feces into the distal colon via enema, or instillation in the entire colon via colonoscopy. Numerous Phase 2 studies on safety and efficacy for FMT can be found on ClinicalTrials.gov. Only one study has compared the safety and efficacy of various routes; the authors compared FMT delivery via colonoscopy and nasogastric tube and reported no difference in efficacy of

preventing recurrent CDI.[55] Future research should focus on adequately powered, controlled, and blinded RCTs assessing FMT, including patients requiring systemic antibiotics and concomitant antibiotics, and incorporate long-term followup; several such studies are already registered. (Appendix Table I1)

Further research is also needed for probiotics. This is a challenging topic, since the human gut ecology is a complex system. Since food and beverages can also be significant sources of probiotics, establishing clear comparator groups can be difficult. Bakken's case series suggests patient preferences for probiotics (in this case, kefir beverages) and tapered antibiotics before resorting to (or being able to afford) FMT for patients with recurrent CDI suggests a more nuanced understanding of patient preferences and appropriate targets to support healthy digestive and immune systems would be useful.[72] Further information generated by the human biome research initiative may help inform this area.

A randomized trial of different therapies for refractory patients, including FMT, would also advance the field.

Limitations

This review has several limitations. In keeping with the original review, most diagnostic studies included in this update enrolled samples from patients at risk for or with symptoms consistent with CDI. However, some studies included unformed specimens only regardless of whether testing for CDI was requested by the patient's clinician. Studies generally did not describe the clinical characteristics of the patients from whom fecal samples were obtained for inclusion, making it difficult to determine the applicability of findings. Further, we could not determine the impact of enrolling nonconsecutive samples on the measured operating characteristics of a certain diagnostic test. We cannot exclude the possibility that a study with nonconsecutive sample of patients could systematically entrain bias if there were characteristics that led to samples being included and others excluded, such as volume of stool, variability of testing practices in certain wards, or other characteristics.

Requiring patient-centered outcomes such as CDI incidence for prevention studies resulted in the exclusion of several transmission interruption studies. Some decisionmakers may be willing to use studies that examined intermediate outcomes, such as the number of cultures obtained from swabs. However, we encountered no literature directly tying numbers of cultures to actual CDI incidence and thus could not infer clinical meaning from a reduction in cultured swabs.

Pooling diagnostic tests by test class resulted in heterogeneity for most test classes. We examined the heterogeneity for pooled individual tests with sufficient numbers of studies and found significant heterogeneity in these meta-analyses as well. Thus, we deemed the gain in information by pooling test classes worth the cost of the added uncertainty from the heterogeneity.

Conversely, pooled meta-analyses for probiotics studies showed low heterogeneity, even though we pooled liberally based on the probiotic strain(s) included in each study. Pooling used a conceptual basis, representing only one possible way of categorizing the probiotic interventions. Due to lack of subgroup information, we were unable to conduct subgroup analysis on populations at different risk for CDI, in particular patients over age 65.

Conclusion

This update systematically reviewed and assessed the evidence for diagnosis, prevention, and treatment of *C. difficile* using the original report and newly available evidence. While all of the

Key Questions had new literature to incorporate, the research on diagnostic testing for and interventions to treat CDI expanded considerably in 4 years. The review update allowed for 7 new findings, updated 6 findings from the original review, while 20 findings remained essentially unchanged. Overall, several findings are of particular note. Nucleic acid amplification tests have high sensitivity and specificity for CDI. Vancomycin is more effective than metronidazole for initial CDI, while fidaxomicin is more effective than vancomycin for the prevention of recurrent CDI. FMT and lactobacillus probiotics to restore colonic biodiversity and improve patient resistance to CDI or recurrence have low strength but relatively consistent positive evidence for efficacy. There are many possible avenues for future research to improve our understanding of effective diagnostic testing, prevention, and treatment of CDI.

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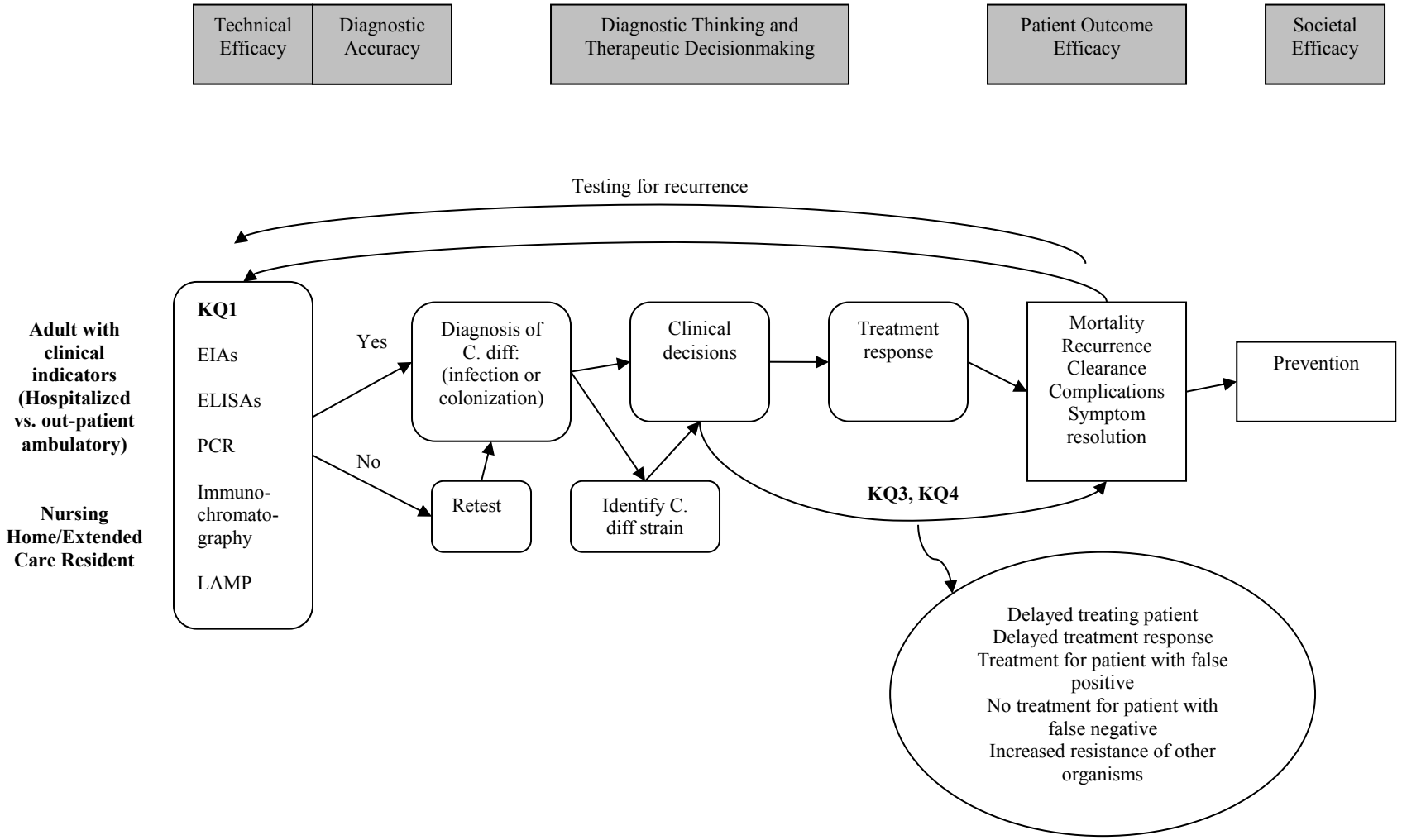
Abbreviations

AHRQ	Agency for Healthcare Research and Quality
CCNA	Cell cytotoxicity neutralization assay
CDI	*Clostridium difficile* infection
CENTRAL	Cochrane Central Register of Controlled Trials
CER	Comparative Effectiveness Review
CI	Confidence interval
FMT	Fecal microbiota transplantation
GDH	Glutamate dehydrogenase
GI	Gastrointestinal
ICTRP	International Controlled Trials Registry Platform
IDSA	Infectious Disease Society of America
IND	Investigational New Drug
KQ	Key Question
LAMP	Loop mediated isothermal amplification
MRSA	Methicillin-resistant staphylococcus aureus
NAAT	Nucleic acid amplification tests
PCR	Polymerase chain reaction
PICOTS	Population, Interventions, Comparators, Outcomes, Timing, Settings
RCT	Randomized controlled trial
RD	Risk difference
ROC	Receiver operating characteristic
RR	Risk ratio
SIP	Scientific information packet
SMD	Standard mean difference
WMD	Weighted mean difference

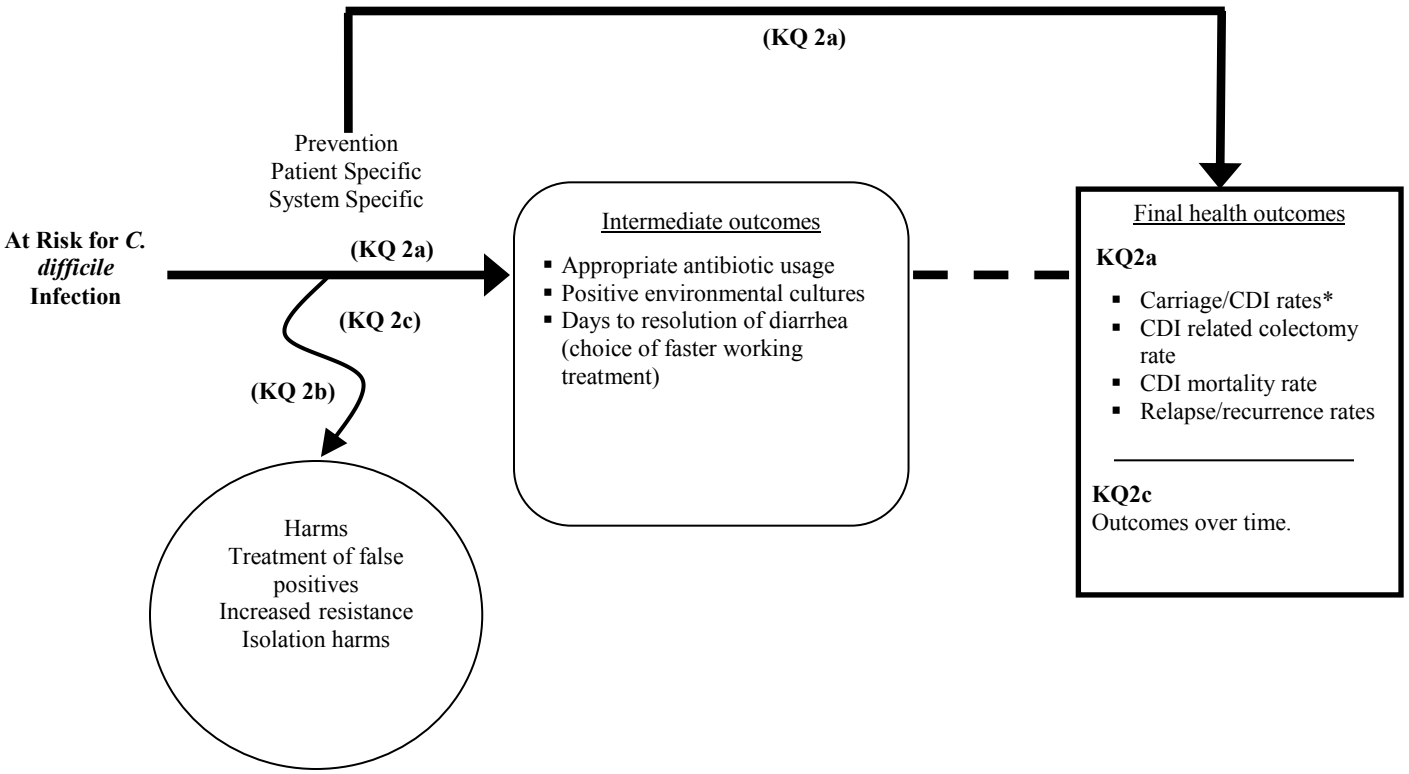
Appendixes

Appendix A. Analytic Frameworks

Appendix Figure A1. Framework for diagnostic testing and treatment



Appendix Figure A2. Analytic framework for CDI prevention



Appendix B. Search Strings

Search String for Diagnostics (not filtered for study design)

1 difficile.mp.
2 limit 1 to (english language and humans)
3 (animals not (humans and animals)).sh.
4. 2 not 3
5 limit 4 to (addresses or bibliography or biography or dictionary or directory or duplicate publication or editorial or interview or introductory journal article or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or portraits)
6. 4 not 5

Appendix C. Excluded Studies

(reason for exclusion appears in italics after each reference)

Key Question 1

1. Agaronov M, Karak SG, Maldonado Y, et al. Comparison of GeneXpert PCR to BD GeneOhm for detecting C. difficile toxin gene in GDH positive toxin negative samples. Annals of Clinical & Laboratory Science 2012; 42(4):397-400. PMID: 23090736. *indeterminate samples only*
2. Baker I, Leeming JP, Reynolds R, et al. Clinical relevance of a positive molecular test in the diagnosis of Clostridium difficile infection. Journal of Hospital Infection 2013 Aug; 84(4):311-5. PMID: 23831282. *ref standard not applied to all samples*
3. Barbut F, Surgers L, Eckert C, et al. Does a rapid diagnosis of Clostridium difficile infection impact on quality of patient management? Clinical Microbiology & Infection 2014 Feb; 20(2):136-44. PMID: 23565919. *inadequate reference standard*
4. Beck ET, Buchan BW, Riebe KM, et al. Multicenter evaluation of the Quidel Lyra Direct C. difficile nucleic acid amplification assay. Journal of Clinical Microbiology 2014 Jun; 52(6):1998-2002. PMID: 24671790. *test component only*
5. Behroozian AA, Chludzinski JP, Lo ES, et al. Detection of mixed populations of Clostridium difficile from symptomatic patients using capillary-based polymerase chain reaction ribotyping. Infection Control & Hospital Epidemiology 2013 Sep; 34(9):961-6. PMID: 23917911. *typing only*
6. Berry N, Sewell B, Jafri S, et al. Real-time polymerase chain reaction correlates well with clinical diagnosis of Clostridium difficile infection. Journal of Hospital Infection 2014 Jun; 87(2):109-14. PMID: 24795170. *inadequate reference standard*
7. Bomers MK, Menke FP, Savage RS, et al. Rapid, Accurate, and On-Site Detection of C. difficile in Stool Samples. American Journal of Gastroenterology 2015 Apr; 110(4):588-94. PMID: 25823766. *standard samples*
8. Boyanton BL, Jr., Sural P, Loomis CR, et al. Loop-mediated isothermal amplification compared to real-time PCR and enzyme immunoassay for toxigenic Clostridium difficile detection. Journal of Clinical Microbiology 2012 Mar; 50(3):640-5. PMID: 22189114. *ref standard not applied to all samples*
9. Catanzaro M, Cirone J. Real-time polymerase chain reaction testing for Clostridium difficile reduces isolation time and improves patient management in a small community hospital. American Journal of Infection Control 2012 Sep; 40(7):663-6. PMID: 22153847. *ref standard not applied to all samples*
10. Chapin KC, Dickenson RA, Wu F, et al. Comparison of five assays for detection of Clostridium difficile toxin. Journal of Molecular Diagnostics 2011 Jul; 13(4):395-400. PMID: 21704273. *ref standard not applied to all samples*
11. Church DL, Chow BL, Lloyd T, et al. Evaluation of automated repetitive-sequence-based PCR (DiversiLab) compared to PCR ribotyping for rapid molecular typing of community- and nosocomial-acquired Clostridium difficile. Diagnostic Microbiology & Infectious Disease 2011 Jun; 70(2):183-90. PMID: 21596222. *typing only*
12. Cohen J, Limbago B, Dumyati G, et al. Impact of changes in Clostridium difficile testing practices on stool rejection policies and C. difficile positivity rates across multiple laboratories in the United States. Journal of Clinical Microbiology 2014 Feb; 52(2):632-4. PMID: 24478500. *ref standard not applied to all samples*
13. Deak E, Miller SA, Humphries RM. Comparison of Illumigene, Simplexa, and AmpliVue Clostridium difficile molecular assays for diagnosis of C. difficile infection. Journal of Clinical Microbiology 2014 Mar; 52(3):960-3. PMID: 24352999. *ref standard not applied to all samples*

14. Denys GA. Portrait Toxigenic Clostridium difficile assay, an isothermal amplification assay detects toxigenic C. difficile in clinical stool specimens. Expert Review of Molecular Diagnostics 2014 Jan; 14(1):17-26. PMID: 24308336. *not study design*

15. Deshpande A, Pasupuleti V, Patel P, et al. Repeat stool testing for Clostridium difficile using enzyme immunoassay in patients with inflammatory bowel disease increases diagnostic yield. Current Medical Research & Opinion 2012 Sep; 28(9):1553-60. PMID: 22852871. *ref standard not applied to all samples*

16. Dionne LL, Raymond F, Corbeil J, et al. Correlation between Clostridium difficile bacterial load, commercial real-time PCR cycle thresholds, and results of diagnostic tests based on enzyme immunoassay and cell culture cytotoxicity assay. Journal of Clinical Microbiology 2013 Nov; 51(11):3624-30. PMID: 23966497. *standard samples*

17. Doing KM, Hintz MS. Prospective evaluation of the Meridian Illumigene loop-mediated amplification assay and the Gen Probe ProGastro Cd polymerase chain reaction assay for the direct detection of toxigenic Clostridium difficile from fecal samples. Diagnostic Microbiology & Infectious Disease 2012 Jan; 72(1):8-13. PMID: 22015321. *ref standard not applied to all samples*

18. Dubberke ER, Han Z, Bobo L, et al. Impact of clinical symptoms on interpretation of diagnostic assays for Clostridium difficile infections. Journal of Clinical Microbiology 2011 Aug; 49(8):2887-93. PMID: 21697328. *patients not randomly or consecutively selected*

19. Eckert C, Burghoffer B, Lalande V, et al. Evaluation of the chromogenic agar chromID C. difficile. Journal of Clinical Microbiology 2013 Mar; 51(3):1002-4. PMID: 23269743. *culture study only*

20. Eckert C, Van Broeck J, Spigaglia P, et al. Comparison of a commercially available repetitive-element PCR system (DiversiLab) with PCR ribotyping for typing of clostridium difficile strains. Journal of Clinical Microbiology 2011 Sep; 49(9):3352-4. PMID: 21775548. *typing only*

21. Freifeld AG, Simonsen KA, Booth CS, et al. A new rapid method for Clostridium difficile DNA extraction and detection in stool: toward point-of-care diagnostic testing. Journal of Molecular Diagnostics 2012 May-Jun; 14(3):274-9. PMID: 22402170. *ref standard not applied to all samples*

22. Goldenberg SD, Dieringer T, French GL. Detection of toxigenic Clostridium difficile in diarrheal stools by rapid real-time polymerase chain reaction. Diagnostic Microbiology & Infectious Disease 2010 Jul; 67(3):304-7. PMID: 20542211. *patients not randomly or consecutively selected*

23. Grein JD, Ochner M, Hoang H, et al. Comparison of testing approaches for Clostridium difficile infection at a large community hospital. Clinical Microbiology & Infection 2014 Jan; 20(1):65-9. PMID: 23521523. *inadequate reference standard*

24. Guerrero DM, Chou C, Jury LA, et al. Clinical and infection control implications of Clostridium difficile infection with negative enzyme immunoassay for toxin. Clinical Infectious Diseases 2011 Aug 1; 53(3):287-90. PMID: 21765078. *ref standard not applied to all samples*

25. Gyorke CE, Wang S, Leslie JL, et al. Evaluation of Clostridium difficile fecal load and limit of detection during a prospective comparison of two molecular tests, the illumigene C. difficile and Xpert C. difficile/Epi tests. Journal of Clinical Microbiology 2013 Jan; 51(1):278-80. PMID: 23052320. *inadequate reference standard*

26. Han Z, McMullen KM, Russo AJ, et al. A Clostridium difficile infection "intervention": change in toxin assay results in fewer C difficile infection cases without changes in patient outcomes. American Journal of Infection Control 2012 May; 40(4):349-53. PMID: 21794950. *ref standard not applied to all samples*

27. Hernandez-Rocha C, Barra-Carrasco J, Alvarez-Lobos M, et al. Prospective comparison of a commercial multiplex real-time polymerase chain reaction and an enzyme immunoassay with toxigenic culture in the diagnosis of Clostridium difficile-associated infections. Diagnostic Microbiology & Infectious Disease 2013 Apr; 75(4):361-5. PMID: 23415540. *ref standard not applied to all samples*

28. Huang B, Jin D, Zhang J, et al. Real-time cellular analysis coupled with a specimen enrichment accurately detects and quantifies Clostridium difficile toxins in stool. Journal of Clinical Microbiology 2014 Apr; 52(4):1105-11. PMID: 24452160. *non-standard test*

29. Ingle M, Deshmukh A, Desai D, et al. Clostridium difficile as a cause of acute diarrhea: a prospective study in a tertiary care center. Indian Journal of Gastroenterology 2013 May; 32(3):179-83. PMID: 23526401. *ref standard not applied to all samples*

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31. Kamboj M, Babady NE, Marsh JW, et al. Estimating risk of C. difficile transmission from PCR positive but cytotoxin negative cases. PLoS ONE [Electronic Resource] 2014; 9(2):e88262. PMID: 24523882. *pediatric patients*

32. Karre T, Sloan L, Patel R, et al. Comparison of two commercial molecular assays to a laboratory-developed molecular assay for diagnosis of Clostridium difficile infection. Journal of Clinical Microbiology 2011 Feb; 49(2):725-7. PMID: 21123537. *ref standard not applied to all samples*

33. Khanna S, Pardi DS, Rosenblatt JE, et al. An evaluation of repeat stool testing for Clostridium difficile infection by polymerase chain reaction. Journal of Clinical Gastroenterology 2012 Nov-Dec; 46(10):846-9. PMID: 22334221. *ref standard not applied to all samples*

34. Koo HL, Van JN, Zhao M, et al. Real-time polymerase chain reaction detection of asymptomatic Clostridium difficile colonization and rising C. difficile-associated disease rates. Infection Control & Hospital Epidemiology 2014 Jun; 35(6):667-73. PMID: 24799643. *not study design*

35. LaSala PR, Svensson AM, Mohammad AA, et al. Comparison of analytical and clinical performance of three methods for detection of Clostridium difficile. Archives of Pathology & Laboratory Medicine 2012 May; 136(5):527-31. PMID: 22540301. *ref standard not applied to all samples*

36. Leibowitz J, Soma VL, Rosen L, et al. Similar proportions of stool specimens from hospitalized children with and without diarrhea test positive for Clostridium difficile. Pediatric Infectious Disease Journal 2015 Mar; 34(3):261-6. PMID: 25247582. *pediatric patients*

37. Leis JA, Gold WL, Ng J, et al. Indeterminate tcdB using a Clostridium difficile PCR assay: a retrospective cohort study. BMC Infectious Diseases 2013; 13:324. PMID: 23865713. *indeterminate samples only*

38. Leslie JL, Cohen SH, Solnick JV, et al. Role of fecal Clostridium difficile load in discrepancies between toxin tests and PCR: is quantitation the next step in C. difficile testing?.[Erratum appears in Eur J Clin Microbiol Infect Dis. 2012 Dec;31(12):3301]. European Journal of Clinical Microbiology & Infectious Diseases 2012 Dec; 31(12):3295-9. PMID: 22814877. *not study design*

39. Liu C, Jiang DN, Xiang GM, et al. DNA detection of Clostridium difficile infection based on real-time resistance measurement. Genetics & Molecular Research 2013; 12(3):3296-304. PMID: 24065671. *standard samples*

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41. Luk S, To WK, Ng TK, et al. A cost-effective approach for detection of toxigenic Clostridium difficile: toxigenic culture using ChromID Clostridium difficile agar. Journal of Clinical Microbiology 2014 Feb; 52(2):671-3. PMID: 24478510. *non-standard test*

42. Luna RA, Boyanton BL, Jr., Mehta S, et al. Rapid stool-based diagnosis of Clostridium difficile infection by real-time PCR in a children's hospital. Journal of Clinical Microbiology 2011 Mar; 49(3):851-7. PMID: 21209161. *pediatric patients*

43. McAuliffe GN, Anderson TP, Stevens M, et al. Systematic application of multiplex PCR enhances the detection of bacteria, parasites, and viruses in stool samples. Journal of Infection 2013 Aug; 67(2):122-9. PMID: 23603249. *ref standard not applied to all samples*

44. McElgunn CJ, Pereira CR, Parham NJ, et al. A low complexity rapid molecular method for detection of Clostridium difficile in stool. PLoS ONE [Electronic Resource] 2014; 9(1):e83808. PMID: 24416173. *non-standard test*

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47. Murad YM, Perez J, Nokhbeh R, et al. Impact of polymerase chain reaction testing on Clostridium difficile infection rates in an acute health care facility. American Journal of Infection Control 2015 Apr 1; 43(4):383-6. PMID: 25687359. *inadequate reference standard*

48. Naaber P, Stsepetova J, Smidt I, et al. Quantification of Clostridium difficile in antibiotic-associated-diarrhea patients. Journal of Clinical Microbiology 2011 Oct; 49(10):3656-8. PMID: 21865427. *non-standard test*

49. Nolte FS, Ribeiro-Nesbitt DG. Clinical comparison of Simplexa universal direct and BD GeneOhm tests for detection of toxigenic Clostridium difficile in stool samples. Journal of Clinical Microbiology 2014 Jan; 52(1):281-2. PMID: 24197886. *inadequate reference standard*

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Key Question 2

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Key Question 3

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Key Question 4

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7. Ting LSL, Praestgaard J, Grunenberg N, et al. A first-in-human, randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose study to assess the safety and tolerability of LFF571 in healthy volunteers. Antimicrobial Agents and Chemotherapy 2012 November; 56(11):5946-51. PMID: 2012631196. *not included intervention*
8. Tvede M, Tinggaard M, Helms M. Rectal bacteriotherapy for recurrent Clostridium difficile-associated diarrhoea: results from a case series of 55 patients in Denmark 2000-2012. Clinical Microbiology & Infection 2015 Jan; 21(1):48-53. PMID: 25636927. *not included intervention*
9. Vickers R, Robinson N, Best E, et al. A randomised phase 1 study to investigate safety, pharmacokinetics and impact on gut microbiota following single and multiple oral doses in healthy male subjects of SMT19969, a novel agent for Clostridium difficile infections. BMC Infectious Diseases 2015; 15(1):91. PMID: 25880933. *not included intervention*

Appendix D. Risk-of-Bias Assessment Form for Observational Studies

***Author*_____ *Year*_____ *(PMID)*_____ *Reviewer*_____**

Question	Response	Criteria	Justification
	Internal Validity		
1. Is the study design prospective, retrospective, or mixed?	Prospective ☐	Outcome has not occurred at the time the study is initiated and information is collected over time to assess relationships with the outcome.	
	Mixed ☐	Studies in which one group is studied prospectively and the other retrospectively.	
	Retrospective ☐	Analyzes data from past records.	
2. Are inclusion/exclusion criteria clearly stated?	Yes ☐		
	Partially ☐	Some, but not all, criteria stated or some not clearly stated.	
	No ☐		
3. Are baseline characteristics measured using valid and reliable measures and equivalent in both groups?	Yes ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained.	
4. Is the level of detail describing the intervention adequate?	Yes ☐	Intervention described included adequate service details	
	Partially ☐	Some of the above features.	
	No ☐	None of the above features.	
5. Is the selection of the comparison group appropriate?	Yes ☐	Considering diagnostic assessment, other patient characteristics	
	Partially ☐		
	No ☐		
6. Did researchers isolate the impact from a concurrent intervention or an unintended exposure that might bias results?	Yes ☐	Accounted for concurrent informal care.	
	Partially ☐		
	No ☐		
7. Any attempt to balance the allocation between the groups (e.g., stratification, matching, propensity scores)?	Yes ☐	(If yes, what was used?)	
	No ☐		
	Uncertain ☐	Could not be ascertained.	
8. Were outcomes assessors blinded?	Yes ☐	Who were outcome assessors?	
	No ☐		
9. Are outcomes assessed using valid and reliable measures, implemented	Yes ☐	Measure valid and reliable (i.e., objective measures, well validated scale, provider report); and equivalent across groups.	

Question	Response	Criteria	Justification
consistently across all study participants?	Partially ☐	Some of the above features (partially validated scale)	
	No ☐	None of the above features (self-report, scales with lower validity, reliability); not equivalent across groups	
	Uncertain ☐	Could not be ascertained.	
10. Is the length of followup the same for all groups?	Yes ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained.	
11. Did attrition result in a difference in group characteristics between baseline and followup?	Yes ☐	(Measurement period of interest if repeated measures)	
	No ☐		
	Uncertain ☐	Could not be ascertained (i.e. retrospective designs where eligible at baseline could not be determined)	
12. If baseline characteristics are not similar, does the analysis control for baseline differences between groups?	Yes ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained (i.e., retrospective designs where eligible at baseline could not be determined)	
13. Are confounding and/or effect modifying variables assessed using valid and reliable measures across all study participants?	Yes ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained (i.e., retrospective designs where eligible at baseline could not be determined)	
	NA ☐	No confounders or effect modifiers included in the study.	
14. Were the important confounding and effect modifying variables taken into account in the design and/or analysis (e.g., through matching, stratification, interaction terms, multivariate analysis, or other statistical adjustment)?	Yes ☐		
	Partially ☐	Some variables taken into account or adjustment achieved to some extent.	
	No ☐	Not accounted for or not identified.	
	Uncertain ☐	Could not be ascertained	
15. Are the statistical methods used to assess the primary outcomes appropriate to the data?	Yes ☐	Statistical techniques used must be appropriate to the data.	
	Partially ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained	

Question	Response	Criteria	Justification
16. Are reports of the study free of suggestion of selective outcome reporting?	Yes ☐		
	No ☐	Not all prespecified outcomes reported, subscales not prespecified reported, outcomes reported incompletely.	
	Uncertain ☐	Could not be ascertained.	
17. Funding source identified	No ☐		Industry, government, university, Foundation (funded by what money source?)
	Yes ☐	Who provided funding?	
	Uncertain ☐		
	Overall Assessment		
18. Overall Risk of Bias assessment	Low ☐	Results are believable taking study limitations into consideration	
	Moderate ☐	Results are probably believable taking study limitations into consideration	
	High ☐	Results are uncertain taking study limitations into consideration	

Appendix E. Description and Characteristics of Included Studies

KQ1 – Diagnostics

Appendix Table E1. Included diagnostics

Study Author	Country	Single or Multicenter	Sample	Patient Population	Number of Samples	N (patients)
Alcala 2015[1]	Spain	Single	Unformed	From October 2012 to March 2013, all loose stool specimens sent to the laboratory of the Hospital General Universitario Gregorio Marañón (Madrid, Spain) for CDI diagnosis were tested in parallel with the direct cytotoxicity assay, toxigenic culture, and the two multistep algorithms evaluated.	979	799
Barkin, 2012[2]	U.S.	Single	Unformed	At least 18, able to enroll, had diarrhea defined as three or more bowel movements in 24 hours, had stool sample submitted for CDI testing per clinician discretion and fulfilled one or more criteria for increased risk of CDI. Prior history of CDI, nosocomial exposure in last 6 months, antibiotic PPI use within previous 3 months, age 65 or older or the presence of nasogastric or postpyloric feeding tube. Subjects exluded if currently being treated for documented CDI and then re-tested during study period. 80 men and 59 women.	272	139
Bruins, 2012[3]	Netherlands	Single	Unformed	All unformed stool samples sent to our laboratory from hospitalized and unhospitalized patients with diarrhea, preferably those known to have CDI-associated symptoms or risk factors such as the recent use of antibiotics, were included in the study	986	NA
Buchan, 2012[4]	U.S.	Multicenter	Unformed	Patients suspected of having C. difficile-associated diarrhea and patients suspected of having C. difficile-associated diarrhea were collected	540	540
Calderaro, 2012[5]	Italy	Single	Not specified	Patients attending the University Hospital of Parma (Northern Italy) with a suspicion of CDI	306	306
Carroll, 2013[6]	U.S.	Multicenter	Unformed	Included in the study were leftover deidentified stool samples submitted to the clinical laboratory specifically for C. difficile testing according to the institution's routine practices.	1,875	1,875
Dalpke, 2013[7]	Germany	Single	Unformed	Patients at the University Hospital Heidelberg between April and July 2012	448	333
de Boer, 2010[8]	Netherlands	Single	Unformed	Three different panels of stool specimens were collected. One panel of 20 stool samples, which differed in consistency (unformed towatery, diarrhoeal), was collected at the Laboratory for Infectious Diseases. The second panel consisted of 161 clinical stool specimens from patients for whom a specific request for CDI was issued. The third panel a	161	NA

Study Author	Country	Single or Multicenter	Sample	Patient Population	Number of Samples	N (patients)
				subset of 32 C. difficile toxigenic culture positive stool samples, that were part of a sample collection described previously		
de Jong, 2012[9]	Netherlands	Single	Unformed	A total of 150 patients were included during a 2-month period, of which 49.7% were male and the median age was 61 years (range 19–95). Most patients were admitted to the medical wards (56%), followed by the surgical (20.7%) and hematology/oncology wards (20.7%) and the intensive care units (2.6%)	150	150
Eckert, 2014[10]	France	Multicenter	Unformed	Patients suspected of having CDI and hospitalized in one of four different university-affiliated hospitals in Paris (Saint-Antoine, Tenon, Trousseau, and Rothschild hospitals). Only diarrheic stool samples were included. C. difficile testing was done in the case of a specific request from the physician and systematically in all cases of nosocomial diarrhea (occurring after day 3 of hospitalization)."	308	NA
Eigner, 2014[11]	Germany	Multicenter	Unformed	Suspected CDI	250	NA
Herrera, 2010[12]	Mexico	Single	Not specified	All samples sent for detection of C. difficile toxins to the Laboratory of Clinical Microbiology	230	NA
Hirvonen, 2013[13]	Finland	Single	Unformed	Inpatients with antibiotic associated diarrhea, ages 7-95	310	310
Hoegh, 2012[14]	Denmark	Single	Not specified	Patients at Hvidovre Hospital having routine testing for C. diff	704	631
Humphries, 2013[15]	U.S.	Single	Unformed	Adult inpatients were included in this study if they had a liquid stool specimen submitted to the clinical microbiology laboratory for C. difficile testing. All patients with a positive NAAT in the study were matched with an equal number of patients with negative NAAT results daily.	296	296
Jensen, 2014[16]	Denmark	Single	Not specified	Criteria for testing was infectious diarrhea	300	283
Kim, 2012[17]	Korea	Single	Unformed	Severance hospital patients with diarrheal stool specimens submitted for testing.	127	127
Knetsch, 2011[18]	UK	Single	Unformed	Diarrheal samples submitted to the Department of Microbiology at Leeds Teaching Hospitals	526	NA
Lalande, 2011[19]	France	Single	Unformed	Patients suspected of having CDIs	472	472
Landry, 2014[20]	U.S.	Single	Unformed	Patients at Yale-New Haven Hospital, samples submitted for C. difficile testing	338	300
Le Guern, 2012[21]	France	Single	Unformed	Inpatients. Criteria for rejection included formed stools or a duplicate specimen submitted during the last 7 days.	360	360
Leitner, 2013[22]	Austria	Single	Unformed	Patients of both genders with specified request for clarification of CDI were tested, 65 males with an age range of 1-88 years and 115 females with age range 2-92 years.	180	180
Mattner, 2012[23]	Germany	Single	Unformed	Liquid stool samples sent to a university microbiology laboratory were investigated for toxigenic C. difficile	256	256

Study Author	Country	Single or Multicenter	Sample	Patient Population	Number of Samples	N (patients)
Noren, 2011[24]	Sweden	Multicenter	Not specified	Consecutive stool specimens submitted for C. difficile diagnostics from hospitals and communities in Orebro County, Sweden, ages 3 months to 96 years	272	272
Noren, 2014[25]	Sweden	Single	Not specified	Patients with clinical signs of CDI admitted to Hoglandet Hospital Eksjo and/or visited primary health care facilities	302	302
Planche, 2013[26]	UK	Multicenter	Unformed	Faecal samples from both hospital and community patients submitted for routine testing for C difficile. Had diarrhea not clearly attributable to an underlying disease or treatment from all hospital patients (aged ≥2 years) and from individuals in the community (aged ≥65 years), irrespective of C diffi cile or other testing requests.	12,402	10,186
Putsathit, 2015[27]	Australia	Multicenter	Not specified	Patients from 3 hospitals in Australia	334	NA
Qutub, 2011[28]	Saudi Arabia	Single	Not specified	Patients admitted and suspected to have CDAD were evaluated, with majority of these patients having had received different types of antibiotics, including third generation of cephalosporins, quinolones, and macrolides.	150	150
Reller, 2010[29]	U.S.	Single	Unformed	Sequential weekday stool samples submitted for suspected C difficile	600	600
Rene, 2011[30]	Canada	Single	Unformed	Consecutive liquid fecal samples from unique patients submitted for routine CCNA	494	494
Shin, 2012[31]	Korea	Multicenter	Not specified	Patients with clinical signs compatible with CDI who were hospitalized in 3 teaching hospitals in Seoul City	243	243
Shin, 2012[32]	Korea	Single	Unformed	Patients suspected of having CDI in a tertiary hospital.	253	NA
Strachan, 2013[33]	UK	Single	Formed and unformed	Patient criteria: aged ≥65 years, taking or had recently taken antibiotics, a hospital inpatient, immunosuppressed, requested by the patient's clinician.	860	860
Viala, 2012[34]	France	Single	Unformed	Patients at the Jean Verdier hospital in Paris suburb	94	89
Walkty, 2013[35]	Canada	Multicenter	Unformed	Patients from Health Sciences Centre, St. Boniface Hospital, and Westman suspected of having CDI. Samples were excluded if stool submitted for a patient with a positive C. difficile test result in the preceding 7 days, and samples from patients less than 1 year of age.	428	428
Ylisiurua, 2013[35]	Finland	Multicenter	Unformed	Hospitalized patients with diarrhea, more than half were over the age of 60 years.	884	NA
Zidaric, 2011[36]	Slovenia	Multicenter	Formed and unformed	Hospitalized and nonhospitalized patients suspected of having CDI	194	170

Appendix Table E2. Included diagnostic studies tests

Study Author	Number With CDI	Number Without CDI	Single vs Serial	Reference Standard	Tests
Alcala 2015[1]	117	862	NR	Toxigenic Culture	TechLab QuickChek Complete TechLab QuickChek + GenomEra TechLab QuickChek + Xpert C. difficile
Barkin, 2012[2]	36	236	Both	Toxigenic Culture	Meridian Premier Toxins A & B Microwell EIA Illumigene C. Difficile DNA Amplification Assay ImmunoCard C. difficile
Bruins, 2012[3]	73	913	Single	Toxigenic Culture	ImmunoCard Toxins A & B TechLab QuickChek Complete Premier Toxin A&B Illumigene C. difficile TechLab C Diff Quik Chek GDH
Buchan, 2012[4]	109	431	Single	CCNA and Toxigenic culture	Portrait Toxigenic C. difficile Assay Illumigene C. difficile Xpert C. difficile GeneOhm Cdiff
Calderaro, 2012[5]	88	218	Single	Toxigenic Culture	C. DIFF QUIK CHEK COMPLETE Illumigene assay
Carroll, 2013[6]	275	1600	Single	Toxigenic Culture	Verigene Clostridium difficile Nucleic Acid Assay
Dalpke, 2013[7]	86	362	Single	Toxigenic Culture	BD MAX Cdiff Xpert C. difficile miniVIDAS
de Boer, 2010[8]	16	145	Single	Toxigenic Culture	Xpect C.difficile A/B
de Jong, 2012[9]	17	133	Single	Toxigenic Culture	ImmunoCard Toxin A and B
Eckert, 2014[10]	48	260	NR	Toxigenic Culture (plus 2 more)	GDH+LAMP
Eigner, 2014[11]	77	173	NR	Toxigenic Culture	Premier Toxin A/B BD GeneOhm Xpert C. difficile RidaGene Toxin A/B
Herrera, 2010[12]	13	217	Single	Toxigenic Culture	VIDAS CDA/B ImmunoCard A/B
Hirvonen, 2013[13]	78	232	Single	Toxigenic Culture	GenomEra C. difficile assay
Hoegh, 2012[14]	87	NR	Single	Toxigenic Culture	ImmunoCard Toxins A+B
Humphries, 2013[15]	124	172	Single	Toxigenic Culture	Illumigene C. difficile Premier Toxin A/B
Jensen, 2014[16]	42	241	NR	Toxigenic Culture	Illumigene Xpert C. difficile

Study Author	Number With CDI	Number Without CDI	Single vs Serial	Reference Standard	Tests
Kim, 2012[17]	11	116	Single	Toxigenic Culture	VIDAS C. difficile Toxin A&B AdvanSure RT-PCR
Knetsch, 2011[18]	101	425	Single	Toxigenic Culture	BD GeneOhm Cdiff assay
Lalande, 2011[19]	49	423	Single	Toxigenic Culture	Illumigene C. difficile assay
Landry, 2014[20]	88	212	NR	CCNA or Toxigenic Culture	Simplexa direct PCR
Le Guern, 2012[21]	54	306	Single	Toxigenic Culture	BD Max Cdiff BD GeneOhm Cdiff
Leitner, 2013[22]	23	157	Single	Toxigenic Culture	Premier Toxins A&B BD MAX Cdiff assay
Mattner, 2012[23]	43	213	Single	Toxigenic Culture	Ridascreen toxin A and B
Noren, 2011[24]	50	222	Single	CCNA or Toxigenic culture	LAMP
Noren, 2014[25]	88	214	Single	Toxigenic Culture	Illumigene LAMP Vidas CDAB assay
Planche, 2013[26]	1034	11368	Single	CCNA or Toxigenic culture	Meridian Premier toxins A&B Techlab C diffi cile Tox A/B II Techlab C diff Chek-60 GDH+NAAT Techlab Tox A/B II + NAAT Techlab c-diff chek-60 + Techlab tox A/B II
Putsathit, 2015[27]	24	310	NR	Toxigenic Culture	Techlab C. Diff Check-6 BD Max Cdiff
Qutub, 2011[28]	52	98	Single	CCNA	C. DIFF CHEK60
Reller, 2010[29]	46	554	Single	CCNA	TechLab C. Diff Chek 60 TechLab C. diff Quick Chek TechLab Tox A/B Quik Chek
Rene, 2011[30]	60	435	Single	CCNA or Toxigenic culture	Xpect C. difficile toxin A/B ImmunoCard Toxins A/B TechLab Toxin A/B Quik Chek Premier toxins A&B Prospect C. difficile toxin A/B TechLab QuikChek TechLab Toxin A/B II
Shin, 2012[31]	70	173	Single	Toxigenic Culture	BD GeneOhm Cdiff assay Seeplex Diarrhea-B1 ACE detection assay
Shin, 2012[32]	49	204	Single	Toxigenic Culture	GeneXpert C. diff Assay VIDAS C. difficile A & B assays
Strachan, 2013[33]	98	762	Single	Toxigenic Culture	Premier C. difficile Toxin A & B

Study Author	Number With CDI	Number Without CDI	Single vs Serial	Reference Standard	Tests
Viala, 2012[34]	45	49	Single	Toxigenic Culture	BD GeneOhmCdiff Cepheid XPert C. difficile Illumigene C. difficile
Walkty, 2013[35]	63	365	Single	Toxigenic Culture	TechLab C. Diff Quik Chek TechLab Tox A/B Quik Chek Illumigene assay GDH+Tox A/B GDH+ CCTA GDH+tox A/B +CCTA GDH+illumigene GDH + tox A/B +illumigene
Ylisiurua, 2013[35]	253	631	Single	Toxigenic Culture	RIDASCREEN EIA assay Illumigene LAMP assay RIDA GENE PCR assay
Zidaric, 2011[36]	28	166	Single	Toxigenic Culture	BD GeneOhm Cdiff assay Cepheid Xpert C. difficileassay

KQ2 – Prevention

See Appendix G

KQ3 – Standard Treatment

Appendix Table E3. New included studies standard antibiotic treatments

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control(s) / Study Duration	Outcomes Evaluated
Newly identified trials			
Johnson, 2014[37] Region: Australia, Canada, Europe, United States Funding source: Industry	Population: Hospitalized or ambulatory patients aged ≥18 years with CDI and non–life threatening medical conditions Mean age: 64 % women: 52 Ethnicity: not reported Inclusion criteria: CDI symptoms (≥3 loose stools in 24 hours) and confirmed toxin Severity: mild (3–5 bowel movements BM/day; WBC ≤15 000/mm3; mild or absent abdominal pain due to CDI), moderate (6–9 BM/day; WBC, 15 001–20 000/mm3; mild, moderate, or absent abdominal pain due to CDI); or severe (10 or more BM/day; WBC ≥20 001/mm3; severe abdominal pain due to CDI). Any one of the defining characteristics could have been used to assign a severity category, and the more severe category was used when characteristics overlapped.	N=555 randomized (289 in Study 301, 266 in Study 302) Intervention 1: Vancomycin 125 mg 4 times/day (n=266) Intervention 2: Metronidazole 375 mg 4 times/day (n=289) Treatment duration: 10 days Followup period: 28 days after treatment period	a. Clinical cure, defined as resolution of diarrhea (attainment of bowel movements with a hard or formed consistency on average or 2 or fewer BM/day with a loose or watery consistency on average) and absence of severe abdominal discomfort due to CDI for more than 2 consecutive days including day 10. b. Time to resolution of diarrhea c. Recurrence of CDI, defined as a confirmed CDI diagnosis d. Nonresponse or change in therapy (scored as failure) e. Adverse events
Cornely, 2012[38] Region: Canada, Europe, United States Funding source: Industry	Population: Symptomatic inpatient (68.2%) or outpatient patients age 16 or older Mean age: 63 % women: 61 Ethnicity: not reported Inclusion criteria: Toxins A or B in stool and ≥3 loose stools in 24 hours preceding randomization Severity: severe disease was defined by meeting any of the following: WBC count >15,000 cells/mm^3, serum creatinine >1.5 mg/dL, or temperature >38.5 C	N=535 randomized (509 in modified ITT population); 124 with severe infection (24.4%) Intervention 1: Vancomycin 125 mg 4 times/day (n=257) Intervention 2: Fidaxomicin 200 mg 2 times/day with intervening placebo (n=252) Treatment duration: 10 days Followup period: 28 days	a. Clinical cure, defined as resolution of diarrhea (3 or fewer unformed bowel movements for 2 consecutive days) for the duration of treatment and no further need for treatment as of the 2nd day after the last dose of study drug. A "substantial reduction" in unformed bowel movements but residual mild abdominal discomfort was also considered a clinical cure if no additional therapy was needed within 2 days of treatment completion b. Recurrence, # of patients

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control(s) / Study Duration	Outcomes Evaluated
			(defined as return of 3 or more unformed bowel movements in 24 hours, a positive stool toxin test, and need for retreatment within 30 days of treatment completion) c. Sustained cure (clinical cure without recurrence) d. Adverse events
Newly identified observational study			
Wenisch, 2012[39] Region: Austria Funding source: none received	Population: Hospitalized adults with mild CDI Mean age: 77 % women: 63% Ethnicity: Inclusion criteria: Clinical symptoms of mild CDI (stool frequency <4 times daily and no signs of severe colitis) and microbiological evidence of toxin	N=265 (60 received no treatment and were excluded from analysis) Intervention 1: Metronidazole 500 mg 3 times/day (oral) (n=121) Intervention 2: Metronidazole 500 mg 3 times/day intravenous (n=42) Intervention 3: Vancomycin 250 mg 4 times/day (oral) (n=42)	a. All-cause 30-day mortality b. Relative risk of 30-day mortality after adjustment for sex, age (>65 years), and severity of comorbidity c. Clinical cure d. Clinical recurrence e. Adverse events
Previously identified studies			
Louie 2011[40] Region: Canada, United States Funding source: Industry	Population: Adults with acute symptoms of CDI and a positive result on a stool toxin test Mean age: 62 % women: 56 Inclusion criteria: 16 years of age or older with a diagnosis of CDI, defined by the presence of diarrhea (a change in bowel habits, with >3 unformed bowel movements in the 24-hour period before randomization) and *C. difficile* toxin A, B, or both in a stool specimen obtained within 48 hours before randomization.	N=629 Intervention 1: Fidaxomicin 200 mg 2 times/day (n=302) Intervention 2: Vancomycin 125 mg 4 times/day (n=327) Treatment duration: 10 days Followup period: 30 days	a. Clinical cure, defined by the resolution of diarrhea (i.e., 3 or fewer unformed stools for 2 consecutive days), with maintenance of resolution for the duration of therapy and no further requirement (in the investigator's opinion) for therapy for CDI as of the second day after the end of the course of therapy. b. Clinical recurrence, defined by the reappearance of more than 3 diarrheal stools per 24-hour period within 4 weeks after the cessation of therapy; *C. difficile* toxin A or B, or both, in stool; and a need for retreatment for CDI c. Median time to resolution of

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control(s) / Study Duration	Outcomes Evaluated
			diarrhea d. All-cause mortality e. Adverse events
Zar, 2007[41] Region: United States Funding source: none stated	Population: Mild or severe symptomatic inpatient adults with comorbid conditions Mean age: 58 (47% <60 years) % women: 45 Inclusion criteria: *Clostridium difficile*-associated diarrhea (CDI), testing positive for *C. difficile* cytotoxin Severity: patients with ≥2 points were considered to have severe CDI based on an assessment score developed for this study. One point each was given for age >60 years, temperature >38.3°C, albumin level <2.5 mg/dL, or peripheral WBC count >15,000 cells/mm^3 within 48 hours of enrollment. Two points were given for endoscopic evidence of pseudo-membranous colitis or treatment in the intensive care. All patients had received antimicrobial treatment prior to onset of CDI (>90% within 14 days)	N=172 (mild 54%, severe 46% based on 150 patients completing trial) Intervention 1: Vancomycin (liquid) 125 mg 4 times/day + placebo pill (n=82) Intervention 2: Metronidazole (oral) 250 mg 4 times/day plus placebo liquid (n=90) Treatment duration: 10 days Followup period: 21 days	a. Cure, # of patients (defined as resolution of diarrhea by day 6 of treatment and a negative result of a *C. difficile* toxin A assay at days 6 and 10 of treatment) b. Relapse, # of patients (defined as recurrence of *C. difficile* toxin A-positive diarrhea by day 21 after initial cure) c. All-cause mortality
Wenisch, 1996[42] Region: Austria Funding source: none stated	Population: Symptomatic adults hospitalized for a minimum of 5 days Mean age: 42 % women: 48 Inclusion criteria: age of >18 years and the presence of CDI. Diarrhea was defined as >3 loose stools per day. CDI was diagnosed on the basis of the results of a *C. difficile* toxin assay and/or endoscopic evidence of typical colitis, with the finding of granulocytes in stools	N=126 Intervention 1: Metronidazole 500 mg 3 times/day (n=31) Intervention 2: Fusidic acid 500 mg 3 times/day (n=29) Intervention 3: Vancomycin 500 mg 3 times/day (n=31) Intervention 4: Teicoplanin (injection) 400 mg 2 times/day (n=28) Treatment duration: 10 days Followup period: 30 days	a. Clinical cure, # of patients (defined as no loose stools, gastro-intestinal symptoms, or fever and normalization of serum levels of C-reactive protein and leukocyte counts) b. Clinical failure (defined as persistence of diarrhea after 6 days of treatment c. Clinical relapse (defined as the reappearance of CDI and other symptoms during the followup period) d. Adverse events

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control(s) / Study Duration	Outcomes Evaluated
Teasley, 1983[43] Region: United States Funding source: Veterans Affairs and industry	Population: Symptomatic inpatient adults Mean age: 65 % women: 1 Inclusion criteria: *C difficile*-associated diarrhea and its cytotoxin. All patients had received antimicrobial treatment 14-55 days prior to diarrhea	N=101 Intervention 1: Vancomycin 500 mg 4 times/day (n=56) Intervention 2: Metronidazole 250 mg 4 times/day (n=45) Study duration: 10 days Followup period: 21 days	a. Cure (defined as diarrhea resolved within 6 days of treatment, toleration of complete treatment course, and no relapse in the 21-day followup period) b. Treatment response based diarrhea resolution (defined as <2 stools formed/day) c. Treatment failure (defined as ≤4 loose stools/day after 6 days of treatment. d. Treatment relapse (defined as recurrence with 21 days of diarrhea with ≤4 loose stools/day for a minimum of 2 days)

BM=bowel movements; CDI=*C. difficile* infection; WBC=white blood cell counts

KQ4 – Nonstandard Treatment

Appendix Table E4. Included studies for FMT nonstandard treatments

Author, Year, Country, Design, Funding Source	Population, Age, % Women, Race/ethnicity	Sample Size, Intervention(s), Control(s), Study Duration	Outcomes	Harms
Newly identified studies				
Cammarota, 2015[44] Italy Open-label RCT University	Adults with recurrent CDI (diarrhea [≥3 loose or watery stools per day ≥2 consecutive days, or ≥8 loose stools in 48 hours] and positive CD toxin stool test within 10 weeks of antibiotic treatment), mean age 73, 59% women, race/ethnicity NR	39 FMT: 20 FMT (14 with one infusion of FMT, 6 with >1 infusion) and vancomycin (125 mg four times a day for 3 days); 19 vancomycin only (125 mg four times daily for 10 days, followed by 125–500 mg/day every 2–3 days for ≥3 weeks) Followup: 10 weeks after the end of treatments	Resolution of diarrhea, adverse events	No significant adverse events in either group
Satokari, 2015[45] Finland Retrospective review University, foundation	Adults with recurrent CDI (laboratory-confirmed CDI [positive culture and toxin] despite antimicrobial treatment), mean age 56 (range 20-88), 69% women, race/ethnicity NR	49 FMT (n=23 freeze-stored, n=26 fresh) Followup: 12 weeks (n=49) or 1 year (n=42)	Resolution of diarrhea or symptoms, recurrence, death, adverse events	No serious adverse events, mild transient fever (n=2) after freeze-stored FMT
Zainah, 2015[46] United States Retrospective review Funding NR	Adults hospitalized with severe refractory CDI (severe: endoscopic evidence of pseudomembranous colitis, treatment in the ICU for CDI, or ≥2 of: age >60 years, serum albumin <2.5 mg/dL, temperature >38.3C, WBC count >15,000 cell/mL within 48 hours of CDI diagnosis; refractory: non-resolution of CDI despite 7 days of therapy with oral vancomycin with or without IV metronidazole), mean age 73, 64% women, race/ethnicity NR	14 FMT Followup: 100 days	Resolution of diarrhea, recurrence, death	NR
Dutta, 2014[47] United States Prospective Health organization, University	Adults aged 18-90 with recurrent CDI (≥2 laboratory-confirmed relapses of CDI after antimicrobial treatment), mean age 65 (range 18-89), 82% women, 74% white, 22% black, 4% Asian	27 FMT Followup: mean 21 months (range 10–34)	Resolution of diarrhea or symptoms, CDI, adverse events	Low-grade fever (n=5, 19%), bloating (n=3, 11%), both of which resolved spontaneously within 12–24 hours

Author, Year, Country, Design, Funding Source	Population, Age, % Women, Race/ethnicity	Sample Size, Intervention(s), Control(s), Study Duration	Outcomes	Harms
Khan, 2014[48] United States Retrospective review Funding NR	Adults with recurrent CDI (1-3 courses of metronidazole and/or vancomycin before FMT), mean age 65, 89% women, race/ethnicity NR	20 FMT Followup: 6 months	Resolution of diarrhea, recurrence, adverse events, patient satisfaction	None
Lee, 2014[49] Canada Retrospective review University	Adults with refractory or recurrent CDI (refractory: ongoing diarrhea despite ≥5 days oral vancomycin ≥125 mg 4 times/d; recurrent: symptom resolution ≥2 days after treatment discontinuation with recurrence of diarrhea), mean age 72, 56% women, race/ethnicity NR, 74.5% hospitalized	94 FMT Followup: 6-24 months	Resolution of diarrhea, recurrence, death, adverse events	Transient constipation and excess flatulence (10%)
Ray, 2014[50] Retrospective review Funding NR	Adults with recurrent or severe CDI (≥2 recurrences [>3 loose stools a day or positive CD stool sample after antibiotics] or life-threatening illness from CDI requiring hospitalization and/or ICU admission), mean age 62 (range 27-89), 80% women, race/ethnicity NR	20 FMT: 16 recurrent 3 severe/complicated 1 severe Followup: mean 3 months (range 0-10)	Resolution of diarrhea or symptoms, recurrence, adverse events	Abdominal cramping, bloating, flatulence, nausea that resolved (n=5, 25%)
Seekatz, 2014[51] United States Prospective Government, foundation	Adults with recurrent CDI (≥2 laboratory-confirmed relapses and failure of standard antibiotics), mean age NR, gender NR, race/ethnicity NR	14 FMT Followup: 6 months	Resolution of diarrhea or symptoms, CDI recurrence, adverse events	NR
Weingarden, 2014[52] United States Case series Government, university	Adults with recurrent CDI (3-9 episodes of CDI and failure of multiple rounds of antibiotics), median age 62 (range 29-87), 83% women, race/ethnicity NR	12 FMT Followup: 1 year+	Resolution of diarrhea or symptoms, CDI, recurrence	NR
Youngster 2014[53] United Sates Open-label feasibility study Health organization	Adults with recurrent CDI (≥3 mild to moderate episodes and failure of a 6- to 8-week taper with vancomycin with or without an alternative antibiotic, or ≥2 severe episodes resulting in hospitalization and associated with significant morbidity), median age 65, 45% women, race/ethnicity NR	20 FMT (capsules): 16 recurrent 4 refractory Followup: 6 months	Resolution of diarrhea or symptoms, adverse events	No serious adverse events deemed treatment-related; abdominal cramping and bloating (n=4, 20%)

Author, Year, Country, Design, Funding Source	Population, Age, % Women, Race/ethnicity	Sample Size, Intervention(s), Control(s), Study Duration	Outcomes	Harms
Youngster 2014[54] United States Open-label RCT Government, university	People aged 7-90 with recurrent or refractory CDI (≥3 mild to moderate episodes and failure of a 6- to 8-week taper with vancomycin with or without an alternative antibiotic, or ≥2 severe episodes resulting in hospitalization and associated with significant morbidity), mean age 54 (range 7-90; 3 children), 55% women, race/ethnicity NR	20 FMT: 10 colonoscopic, 10 nasogastric Followup: 8 weeks (n=20), 6 months (n=15)	Resolution of diarrhea without relapse within 8 weeks, adverse events	No serious adverse events; abdominal cramping and bloating (n=6, 30%), which resolved within 72 hours
Emanuelsson, 2014[55] Sweden Retrospective review No funding	Adults with recurrent CDI (failure of repeated courses of antibiotics), median age 69, 61% female, race/ethnicity NR	23 FMT Followup: median 18 months (range 0-201)	Resolution of diarrhea and symptoms, adverse events	No significant adverse events on the day of microbiota infusion
Patel, 2013[56] United States Retrospective review Funding NR	Adults with recurrent CDI (≥2 documented episodes of CDI, failure of antibiotics and ongoing diarrhea [≥3 unformed stools per day] in the absence of antibiotics), mean age 61, 55% women, race/ethnicity NR	31 FMT Followup: 1 week and 1 month (n=30), 3 months (n=23), 1 year (n=6)	Resolution of diarrhea or symptoms, recurrence, death, adverse events	No serious adverse events; microperforation caused by a biopsy during the FMT procedure (n=1)
Pathak, 2014[57] United States Retrospective review Funding NR	Adults with recurrent CDI (≥3 episodes and failure of vancomycin with or without an additional antibiotic; some cases severe but details NR), age range 37-92, 67% women, race/ethnicity NR	12 FMT followed by 2 months of *S. boulardii* Followup: range 2-30 months	Resolution of diarrhea or symptoms	NR
Rubin, 2013[58] United States Retrospective review Health organization	Adults with recurrent CDI (initial laboratory-confirmed diagnosis of CDI and ≥2 laboratory-confirmed recurrences following standard antibiotics), mean age 63, 65% women, race/ethnicity NR	74 FMT Followup: 60 days	Resolution of diarrhea, recurrence, adverse events	None
van Nood, 2013[59] The Netherlands Open-label randomized trial Government	Adults with recurrent CDI (recurrence with positive stool test for CD toxin following at least one adequate course of treatment), mean age 70, 43% women, race/ethnicity NR	43 randomized 17 vancomycin (500 mg 4 times/day for 4 days), bowel lavage, FMT 13 vancomycin, bowel lavage 13 vancomycin Followup: 10 weeks	Resolution of diarrhea, CDI, adverse events	No serious adverse events; immediately after procedure, resolved within 3 hours: diarrhea (94%), cramping (31%), belching (19%); during followup: constipation (19%)

Author, Year, Country, Design, Funding Source	Population, Age, % Women, Race/ethnicity	Sample Size, Intervention(s), Control(s), Study Duration	Outcomes	Harms
Hamilton, 2012[60] United States Case series Foundation, government	Adults with recurrent CDI (history of toxin-positive CDI and ≥2 documented recurrences despite standard antibiotics), mean age 59, 72% women, race/ethnicity NR	43 FMT Followup: NR	Resolution of diarrhea, CDI (not tested if asymptomatic), recurrence, adverse events	No serious adverse events; irregularity of bowel movements and excessive flatulence (approximately one third of patients), which resolved
Jorup-Ronstrom, 2012[61] Sweden Case series Funding NR	Adults with recurrent CDI (≥3 relapses and failure of multiple courses of antibiotics) median age 75 (range 27-94), 62.5% women, race/ethnicity NR	32 FMT (cultured for 10 years) Followup: median 26 months (range 1-68)	Cure ("if no relapse occurred"), improvement, recurrence, adverse events	None
Kelly, 2012[62] United States Case series Funding NR	Adults with recurrent CDI (≥3 relapses and failure of multiple courses of antibiotics), mean age 59 (range 19-86) 92% women, 100% white	26 FMT Followup: mean 11 months (range 2-30)	Resolution of diarrhea, CDI, recurrence	NR
Mattila, 2012[63] Finland Retrospective review Foundation	Adults with recurrent CDI (laboratory-confirmed recurrence [positive culture and toxin] despite antimicrobial treatment), mean age 73 (range 22-90), 60% women, race/ethnicity NR	70 FMT Followup: 12 weeks and 1 year	Resolution of symptoms, recurrence, death, adverse events	No serious adverse events
Mellow, 2011[64] United States Case Series Funding NR	Adults with recurrent (≥3 episodes, n=12) or refractory (not defined, n=1) CDI, mean age 67 (range 32-87), 46% women, race/ethnicity NR	13 FMT Followup: mean 5 months (range 3-24)	Resolution of diarrhea, recurrence, stool test for CDI (n=10), death	NR
Garborg, 2010[65] Norway Retrospective review Funding NR	Adults with recurrent CDI (failure of ≥2 courses of antibiotics; n=37 laboratory-confirmed, n=3 toxin negative), mean age 75 (range 53-94), 53% women, race/ethnicity NR	40 FMT Followup: 80 days (no systematic followup)	Resolution of diarrhea, adverse events	None
Aas, 2003[66] United States Retrospective review Health organization	Adults with recurrent CDI (≥2 laboratory-confirmed relapses following antibiotics), mean age 73 (range 51–88), 72% women, race/ethnicity NR	18 FMT Followup: 90 days	Resolution of diarrhea, stool test for CDI (n=14), recurrence, death, adverse events	None

Author, Year, Country, Design, Funding Source	Population, Age, % Women, Race/ethnicity	Sample Size, Intervention(s), Control(s), Study Duration	Outcomes	Harms
Previously identified studies				
Rohlke, 2010[67] United States Retrospective review No funding	Adults with recurrent CDI (CD toxin positivity and consistently recurring symptoms over ≥6 months, despite ≥3 courses of traditional treatments, including pulsed and tapered vancomycin), mean age 49, 89% women, race/ethnicity NR	19 FMT Followup: mean 27 months (range 6-65)	Resolution of symptoms, recurrence	NR
Yoon, 2010[68] United States Case series No funding	Adults with recurrent CDI (documented CD toxin-positive diarrhea and documented recurrence despite standard antibiotics), mean age 66 (range 30-86), 75% women, race/ethnicity NR	12 FMT Followup: range 3 weeks to 8 years	Resolution of symptoms, adverse events	None
MacConnachie, 2009[69] United Kingdom Retrospective review Funding NR	Adults with recurrent CDI (recurrence of loose stool following antibiotic treatment for toxin positive CDI), mean age 82 (range 68-95), 93% women, race/ethnicity NR	15 FMT Followup: median 16 weeks (range 4-24)	Resolution of symptoms, adverse events	No adverse events related to FMT

CD=*C. difficile*; CDI=*C. difficile* infection; FMT=fecal microbiota transplant; ICU=intensive care unit; NR=not reported; WBC=white blood cell

Appendix Table E5. Included studies for probiotic nonstandard treatments

Author, Year, Country, Funding Source	Population, Age	Sample Size, Intervention(s), Control(s), Study Duration	Adverse Events*
Newly identified randomized trials			
Ouwehand, 2014[70] China Industry	503 adult in-patients aged 30-70 on antibiotic therapy, mean age 50	Four-strain preparation of *L. acidophilus*, *L. paracasei*, and *Bifidobacterium* High-dose, 1.70 x 10^{10} CFU (n = 168) Low-dose, 4.17 x 10^{9} CFU (n = 168) Placebo (n = 167) Treatment duration: 10-21 days; antibiotic duration plus 7 days Followup: 4 weeks after antibiotic course	High-dose: 4.2% Low-dose: 4.2% Placebo: 7.2% allergy to seafood (2), arrhythmia (2), fever (10), headache (2), left upper arm fracture (1), runny nose (4), and vomiting (4)
Allen, 2013[71] United Kingdom Government	2981 adult inpatients aged 65 years and older, mean age 77.2, exposed to one or more parenteral antibiotics	Multistrain preparation of *lactobacilli* and *bifidobacteria*, 6 x 10^{10} organisms for 21 days (n=1493) Placebo (n=1488) Followup: 8 weeks after recruitment, chart review at 12 weeks	No serious adverse events attributed to participation in the trial
Selinger, 2013[72] United Kingdom Industry, government	229 adult hospital inpatients, mean age 58 exposed to systemic antibiotics	VSL#3 probiotic, 450 x 10^{9} cfu/day (n=117) Placebo (n=112) Treatment duration: antibiotic duration plus 7 days Followup: 28 days	Treatment group: 14/117 Placebo: 16/112
Pozzoni, 2012[73] Italy Hospital	275 adult hospital inpatients exposed to antibiotics without ongoing diarrhea or recent use of probiotics, mean age 72	*S. boulardii,* within 48 hours of starting antibiotic therapy (n=141) Placebo (n=134) Treatment duration: antibiotic duration plus 7 days Followup: 12 weeks	Treatment group: 52/141 Placebo: 42/135
Gao, 2010[74] China Industry	255 adult inpatients exposed to antibiotics, aged 50-70, without active diarrhea or CDI within 3 months, mean age 60	*L. acidophilus* CL1285 and *L. casei* LBC80R, 100 x 10^{9} CFU/day (n=86) *L. acidophilus* CL1285 and *L. casei*, LBC80R, 50 x 10^{9} cfu/day (n=85) within 36 hours of starting antibiotic therapy until 5 days after discontinuation; antibiotic duration 3-14 days Placebo (n=85) Followup: 21 days after last study drug dose	Treatment group: 1/171 Placebo: 2/84
Lonnermark, 2010[75] Sweden Funding NR	239 adults (137 inpatients) treated for infections, mean age 45	*L. plantarum* 299v, 10 x 10^{9} cfu/day, within 48 hours of starting antibiotic therapy until 7 days after discontinuation (n=118) Placebo (n=121) Followup: ≥1 week after last study drug dose	Treatment group: 3/80 Placebo: 3/83

Author, Year, Country, Funding Source	Population, Age	Sample Size, Intervention(s), Control(s), Study Duration	Adverse Events*
Psaradellis, 2010[76] Canada Industry	437 adults (248 inpatients) prescribed antibiotics, mean age 59	*L. acidophilus* CL1285 and *L. casei*, 25 x 10^9 CFU/day ,for 2 days then 50 x 10^9 cfu/day until 5 days after discontinuation of antibiotic (n=233) Placebo (n=239) Followup: 21 days after last study drug dose	Treatment group: 87/216 Placebo: 99/221
Safdar, 2008[77] United States Industry NR	40 adult inpatients, elderly U.S. veterans exposed to antibiotics, mean age 69	*L. acidophilus*, 60 x 10^9 cfu/day during and 14 days after antibiotic course (n=23) Placebo (n=17) Followup: NR	Treatment group: 2/23 Placebo: 5/17
Beausoleil, 2007[78] Canada Industry	89 adult inpatients who were anticipated to take systemic antibiotics, mean age 71	*L. acidophilus* CL1285 and *L. casei*, 25 x 10^9 cfu/day for 2 days, then 50 x 10^9 CFU/day for antibiotic duration (n=44) Placebo (n=45) Followup: 21 days after last study drug dose	Treatment group: 21/44 Placebo: 20/45
Duman, 2005[79] Turkey Funding NR	204 adults who received 14 days triple therapy for *Helicobacter pylori* eradication, mean age 45	*S. boulardii*, 30 x 10^9 cfu/day for antibiotic duration (14 days) (n=204) No treatment (n=185) Followup: 4 weeks after last study drug dose	Treatment group: 3/196 No treatment: 4/180
Newly identified observational study			
Maziade, 2013[80] Canada Open prospective Hospital	31,832 hospitalized patients receiving antibiotics, mean age NR	Standard care (n=1580) Standard care plus *L. acidophilus* CL1285 and *L. casei* LBC80R 50-60 × 10^9 cfu/day (n= 4968) Treatment duration: minimum 30 days or antibiotic duration Study duration: 6 years	No serious adverse events
Bakken, 2014[81] Unites States None	25 patients with recurrent CDI	Staggered and tapered antibiotic withdrawal regimen with orgal ingestion of 5 oz probiotic liquid kefir 3 times per day. Followup: up to 1 year	4/25 relapsed within 9 months. No serious adverse events reported.
Previously identified trials			
Hickson, 2007[82] United Kingdom Foundation	135 adult inpatients, mean age 74	*L. casei immunitas* DN-114 001, 19 x 10^9 CFU/day; *L. bulgaris*, 1.9 x 10^9 cfu/day; and *S.thermophiles*, 19 x 10^9 cfu/day within 48 hours of starting antibiotic therapy until 7 days after discontinuation (n=69) Placebo (n=66) Followup: 4 weeks after last antibiotic or study drug dose	Treatment group: 0/56 Placebo: 0/53

Author, Year, Country, Funding Source	Population, Age	Sample Size, Intervention(s), Control(s), Study Duration	Adverse Events*
Can, 2006[83] Turkey Funding NR	151 adult inpatients aged 25-50, mean age NR	*S. boulardii*, lyophilized 20 x 10^9 cfu/day ≤48 hours of antibiotic start dose (duration of study drug course NR) (n=73) Placebo (n=78) Followup: 4 weeks after last antibiotic dose	No serious adverse events
Plummer, 2004[84] United Kingdom Funding NR	150 older adult inpatients	*L. acidophilus* and *Bifidobacterium bifidum*, 20 x 10^9 cfu/day within 36 hours of starting antibiotic therapy, for 20 days (n=69) Placebo (n=69) Followup: Last day of study drug dose	NR
Thomas, 2001[85] United States Industry	302 adult inpatients, mean age 56	*L. rhamnosus* GG, 20 x 10^9 cfu/day within 24 hours of starting antibiotic therapy, for 14 days (n=152) Placebo (n=150) Followup: 7 days after last study drug dose	Treatment group: 37/133 Placebo: 52/134
Lewis, 1998[86] United Kingdom Health organization	72 older adult inpatients, mean age 74 (range 70-85)	*S. boulardii,* 113 mg (n=33) Placebo (n=36)	NR
McFarland, 1995[87] United States Funding NR	193 adult inpatients, mean age 41	*S. boulardii* lyophilized, 30 x 10^9 cfu/day within 72 hours of starting antibiotic therapy until 3 days after discontinuation (n=97) Placebo (n=96) Followup: 7 weeks after last study drug dose	Treatment group: 0/93 Placebo: 12/92
Surawicz, 1989[88] United States Industry	318 adults inpatients (n=138 had CDI tested), mean age 48	*S. boulardii* lyophilized, 20 x 10^9 cfu/day within 48 hours of starting antibiotic therapy until 2 weeks after discontinuation (n=212) Placebo (n=106) Followup: mean 17 days	Treatment group: 0/116 Placebo: 0/64

CDI=C. difficile infection; NR=not reported

* No serious adverse events reported that were attributed to probiotic treatment.

Appendix Table E6. Included RCTs for other nonstandard treatments

Author, Year, Country, Funding Source	Population, Age	Sample Size, Intervention(s), Control(s), Study Duration
Puri, 2015[89]	Adult patients, ages 18-90, diagnosed with primary episode or first recurrence of moderately severe (≥3 to ≤12 liquid or unformed stools in 24 hours prior to enrollment plus one or more abdominal pain, leukocyte count >$10x10^9$/liter but <30, or fever) CDI	LFF571 200 mg (n=46) or oral vancomycin 125 mg (n=26, modified ITT 25) 4 time/day for 10 days Followup: 30 days following 10 day treatment To: clinical cure Non-inferiority trial
Garey, 2011[90]	68 adult inpatients treated for CDI and no longer symptomatic, 50% female, Mean age 61	Rifaximin 400 mg 3 times/day for 20 days immediately after finishing standard anti-CDI antibiotics (n=39 randomized, 33 treated) Placebo (n=40 randomized, 35 treated) Followup: 3 months following 20 day treatment To prevent relapse
Laffan, 2011[91]	30 long-term care facility residents, 64% female, mean age 62, 32%	Recombinant lactoferrin 5mg/mL in 600 mL saline solution for 8 weeks (n=13) Placebo (n=9) (30 participants randomized but initial randomization of the 8 patients excluded from analysis unclear; 6 were from lactoferrin group and 2 were from unknown group) Followup: 14, 42, and 56 days To prevent occurance or relapse

CDI=C. difficile infection

Appendix F. Risk of Bias and Study Quality

KQ1 – Diagnostics

We used an updated rubric for assessing the quality of included studies (QUADAS-2). Overall, 12 of 37 studies were "low risk of bias" in all 4 QUADAS-2 domains (patient selection, index test, reference standard, and flow and timing). In keeping with the previous report, most studies that were included in this report that were not included in the original report enrolled samples from patients at risk for or with symptoms consistent with CDI. However, some studies included enrolled unformed specimens only irrespective of whether testing for CDI was requested by the patient's clinician. The clinical characteristics of the patients from whom fecal samples were obtained for inclusion in the included studies were generally not described, making determination of applicability of findings problematic. While the characteristics of patients from whom fecal specimens were obtained for inclusion in the study were often not described, most studies (26) included only unformed stools samples while two studies contained both formed and unformed specimens and nine studies did not specify whether samples were formed or unformed. Nineteen studies did not include repeat samples from a single patient, but 18 studies included more samples than patients or did not specify the number of patients.

In contrast to the previous report, we included studies that prospectively enrolled samples from a patient population with a "baseline" pre-test probability of CDI without modification of the probability of disease by a screening test. The prevalence of CDI in the studies varied widely, between 6 percent and 48 percent. While this variability may not have an impact on sensitivity and specificity, the positive and negative predictive values of included tests are not applicable to a population with different prevalence than the prevalence of CDI in an included study. Seventeen studies enrolled a random or consecutive sample of samples, 20 studies did not specify if a consecutive or random sample of patients was included, and three studies did not include a random or consecutive sample of specimens. The impact of enrolling nonconsecutive samples on the measured operating characteristics of a certain diagnostic test is unclear. We cannot exclude the possibility that a study that had a nonconsecutive sample of patients could systematically entrain bias if there were characteristics of that led to samples being included and others excluded, such as volume of stool, variability of testing practices in certain wards, or other characteristics.

Similar to the previous report, we found that there were few concerns in the conduct and interpretation of index tests with respect to risk of bias. However, there was significant heterogeneity in the studies and the source of this heterogeneity in observed operating characteristics for the included studies is not completely clear. Many studies did not apply different tests to the exact same number of patients and the reasons for these differences were not often specified. There was some variability in how invalid or inconclusive index test(s) were interpreted and if the index test(s) were repeated on invalid or inconclusive specimens. The previous report included studies with a combination of reference standards including cell cytotoxicity test, cell cytotoxicity test in conjunction with toxigenic culture, one used a toxin immunoassay in conjunction with toxigenic culture, multiple immunoassays for toxins A and B in conjunction with toxigenic culture, and in-house gene detection tests. In the current update report, we used a more stringent reference standard of the cell cytotoxicity assay, toxigenic culture, or a combination thereof. A few studies used enriched toxigenic culture as the reference standard which is likely a more sensitive reference standard that typical toxigenic culture or cytoxicity assay; the logical consequence is that index tests may appear less sensitive when

compared against a more sensitive reference standard. Thirty studies used toxigenic culture as the reference standard, five studies used a composite reference standard of cell cytotoxicity assay and/or toxigenic culture, and two studies used cell cytotoxicity assays as the reference standard. Although regarded as an acceptable reference standard, toxigenic culture, cell cytotoxicity assay or a combination thereof are not perfectly accurate. In the majority of included studies the diagnostic tests were performed independently although it was usually not explicitly stated whether or not the tests were evaluated without knowledge of the other tests. However, it was inferred that most index tests (which are more rapid than the reference standards that take 24-48 hours) were interpreted prior to the results of the reference test being available.

Nineteen studies were "high risk of bias" with respect to flow and timing, mostly due to not all samples being included in the analysis. While the number of indeterminate results was generally small, small changes in a 2x2 table for a certain study can have marked changes in the calculated operating characteristics. As in the previous report, the handling of indeterminate or inconclusive results is problematic. One approach many investigators used was to exclude the inconclusive tests from the calculation of the operating characteristics of a certain test, while others repeated the index test and used the second result (if positive or negative) as the result used in the calculation of operating characteristics. The former approach may lead to an overestimation or underestimation of the sensitivity and specificity of a test depending on whether the reference standard result of the excluded samples is positive or negative. Further, this approach also may lead to the body of samples included being no longer consecutive or random. The latter approach may also lead a misestimation of the operating characteristics as the approach to inconclusive results likely varies significantly between laboratories.

Appendix Table F1. Diagnostic study quality

Author	Year	Patient Selection	Index Test	Reference Standard	Flow and Timing	Test Class Examined
Alcala, 2014[1]	2014	Low	Low	Low	High	GDH, PCR
Barkin, 2012[2]	2012	Low	Low	Low	High	A/B, GDH, LAMP
Bruins, 2012[3]	2012	Low	Unclear	Low	Unclear	PCR, A/B, GDH, LAMP
Buchan, 2012[4]	2012	Unclear	Low	Low	High	LAMP
Calderaro, 2012[5]	2012	Low	Low	Low	Low	GDH, LAMP
Carroll, 2013[6]	2013	Unclear	Low	Low	High	PCR
Dalpke, 2013[7]	2013	Unclear	Low	Low	Low	PCR
de Boer, 2010[8]	2010	Low	Low	Low	Low	PCR
de Jong, 2012[9]	2012	Low	Low	Low	Low	A/B
Eckert, 2014[10]	2014	Low	Low	Low	High	GDH, LAMP
Eigner, 2014[11]	2014	Unclear	Low	Low	High	A/B, PCR
Herrera, 2010[12]	2010	Low	Unclear	Low	Unclear	A/B
Hirvonen, 2013[13]	2013	Low	Low	Low	Low	PCR
Hoegh, 2012[14]	2012	Low	Low	Low	High	A/B
Humphries, 2013[15]	2013	High	Unclear	High	Unclear	A/B, LAMP
Jensen, 2015[16]	2015	Low	Low	Low	High	LAMP, PCR
Kim, 2012[17]	2012	Low	Low	Low	High	PCR, A/B
Knetsch, 2011[18]	2011	Low	Low	Low	High	PCR
Lalande, 2011[19]	2011	Low	Low	Low	Low	LAMP
Landry, 2014[20]	2014	Unclear	Low	Low	Low	PCR
Le Guern, 2012[21]	2012	Low	Low	Low	Low	PCR
Leitner, 2013[22]	2013	Low	Low	Low	Low	PCR, A/B
Mattner, 2012[23]	2012	Low	Low	Low	Low	A/B
Noren, 2011[24]	2011	Low	Low	Low	Low	LAMP
Noren, 2014[25]	2013	Low	Low	Low	High	A/B, LAMP
Planche, 2013[26]	2013	Low	High	Low	High	A/B, GDH, TA
Putsathit, 2014[27]	2014	Unclear	Low	Low	High	GDH, PCR
Qutub, 2011[28]	2011	Low	Low	Low	Low	GDH
Reller, 2010[29]	2010	Unclear	Low	Low	Low	A/B, GDH
Rene, 2011[30]	2012	Low	Low	Low	High	A/B
Shin, 2012[31]	2012	Low	Low	Low	Low	PCR, A/B
Shin, 2012[32]	2012	Low	Low	Low	High	PCR
Strachan, 2013[33]	2013	Low	Low	Low	Low	A/B
Viala, 2012[34]	2012	Low	Low	Low	High	PCR, LAMP
Walkty, 2013[35]	2013	Low	Low	Low	High	A/B, GDH, LAMP, TA
Ylisiurua, 2013[35]	2013	Low	Low	Low	High	PCR, A/B, LAMP
Zidaric, 2011[36]	2011	Low	Low	Low	High	PCR

A/B=toxin A/B test; GDH=glutamate dehydrogenase; LAMP=loop mediated isothermal amplification; PCR-polymerase chain reaction; TA=test algorithm

KQ2 – Prevention

Appendix Table F2. Prevention study risk of bias

Author, Year Country	Study Design	Overall Summary Score	Comments
Transmission Interruption			
Filice, 2013[92] United States	Systematic review	Low	Publication bias not assessed – fewer than 10 relevant studies for CDI outcome. English language only, but focused on interventions in systems similar to the U.S.
Rupp, 2012[93] United States	Quasi-experimental staged introduction trial, 19 months followed by 4 month wash-out	Moderate	Cohorts, not matched concurrent control. Tested for effect of compliance on change in CDI rates. Change in diagnostic testing would conservatively increase CDI rate. Limited information on regression models.
Noto, 2015[94] United States	Pragmatic cluster randomized cross-over trial, 1 year	Moderate	Blinding not possible – outcome assessor blinded. CDI not primary outcome; unlikely powered to capture CDI rate changes.
Alfa, 2015[95] Canada	Interrupted time series single site, 3 years prior, 1 year intervention. prospective	High	Comparator arm (separate location) not used for analysis. Did not control for increase cleaning monitoring.
Levin, 2013[96] United States	Pre/post single site. 1 year followup	High	No concurrent control. Change in antibiotic formulary during study period.
Manian, 2013[97] United States	Retrospective Pre/post single site. 1 year followup	High	No concurrent control
Passaretti 2013[98] United States	Prospective cohort intervention in 3 cohorts. 1 year, 6 month followup	High	No concurrent control. No information on CDI definition.
Stone, 2012[99] United Kingdom	Prospective, ecological, interrupted time series, 3 year followup after roll-out	Moderate	CDI incidence estimated because mandatory reporting database does not include patient age as variable. Limited information on regression models. Analysis reported evidence of selection bias- trusts missing data for soap had lower rates of CDI.
Didiodato, 2013[100] Canada	Prospective, ecological, interrupted time series, 3 year followup	High	Limited information on regression models, imputation for missing data, limited confounding variables captured in the patient safety indicator database.
Bearman, 2010[101] United States	Prospective pre/post single site. 6 month followup	High	No concurrent control
Multicomponent			
Brakovich, 2013[102] United States	Prospective pre/post single site design. 2 year followup	High	No concurrent control
Bishop, 2013[103] United States	Prospective pre/post single site design. 3 year followup	High	No concurrent control
Mermel, 2013[104] United States	Time series single site design. 6 year, 9 month followup	High	Unclear timing for data collection, switch to PCR (more sensitive test) during intervention period confounds change in CDI, limited information on regression model

Author, Year Country	Study Design	Overall Summary Score	Comments
Price, 2010[105] United Kingdom	Interrupted time series single site design; 12 months pre, 15 months post. Retrospective	High	Retrospective data, no information on *C. difficile* diagnostic testing methods, other than no changes except for reduced from 7 to 5 days per week, limited information on regression model.
You, 2014[106] Korea	Retrospective pre/post single site design; 9 months	High	No concurrent control

Appendix Table F3. Quality of previous systematic reviews

Study	A priori Study Design	Dual Study Selection and Data Abstraction	Comprehensive literature search	Publication Status	Lists of Included and Excluded Studies Provided?	Scientific Quality of Included Studies Assessed and Documented?	Scientific Quality of Included Studies Used Appropriately in Formulating Conclusions?	Methods of Combining Studies Appropriate?	Likelihood of Publication Bias Assessed?	Conflict of Interest Stated?	Overall Quality
Filice, 2013[92]	yes	yes	yes	yes	yes	yes	yes	yes	Unclear	yes	good

KQ3 – Standard Treatment

Appendix Table F4. Standard treatment study risk of bias

Study ID	Design	Funding source	Overall Summary	Comments
Johnson, 2014[37]	RCT- 3 arms, tolevamer vs.metronidazole vs. vancomycin	Genzyme (tolevamer maker)	Low risk of bias	No reason to downgrade.
Cornely, 2012[38]	RCT- Vancomycin vs. fidaxomicin	Optimer pharmaceutical (fidaxomicin maker)	Low risk of bias	No reason to downgrade.
Wenisch, 2012[39]	Prospective Cohort – oral metronidazole vs. IV metronidazole vs. oral vancomycin	"No financial support was received for this study"	High risk of bias	Downgraded for: "no" answers to sequence generation, allocation concealment, blinding, and other (non-RCT). Unclear for incomplete outcome data

KQ4 – Nonstandard Treatment

Appendix Table F5. FMT adjunctive treatments study risk of bias

Study Country Funding	Type of Study	Overall Risk of Bias Assessment	Rationale
Newly identified studies			
Cammarota, 2015[44] Italy Nongovernmental	Open-label RCT	High	Unblinded, stopped early, inadequate sample size, change of FMT protocol during study (decided to give multiple infusions after first 2 patients had recurrence after 1 infusion)
Satokari, 2015[45] Finland	Retrospective review	High	Retrospective, case series
Zainah, 2015[46] United States Funding NR	Retrospective review	High	Retrospective, case series, inadequate sample size
Dutta, 2014[47] United States Health organization, University	Prospective	High	Case series, inadequate sample size
Khan, 2014[48] United States Funding NR	Retrospective review	High	Retrospective, case series, inadequate sample size, CDI assessed based on symptoms only, population inclusion criteria (“recurrent CDI”) not defined
Lee, 2014[91] Canada University	Retrospective review	High	Retrospective, case series
Ray, 2014[50] United States Funding NR	Retrospective review	High	Retrospective, case series, inadequate sample size, lack of systematic followup (n=10/20 with 0-1 months followup)
Seekatz, 2014[51] United States Government, foundation	Prospective	High	Case series, inadequate sample size
Weingarden, 2014[52] United States Government, University	Observational	High	Case series, inadequate sample size, population inclusion criteria (“recurrent CDI”) not defined, adverse events not reported
Youngster, 2014[54] United Sates Health organization	Open-label feasibility study	High	Inadequate sample size, no comparison group
Youngster, 2014[53] United States Government, University	Open-label RCT	High	Inadequate sample size, no non-FMT comparison group, attrition
Emanuelsson, 2013[55] Sweden No funding	Retrospective review	High	Retrospective, case series, inadequate sample size, lack of systematic followup (n=5 patients with 0-1 months follow-up)
Patel, 2013[56] United States Funding NR	Retrospective review	High	Retrospective, case series, inadequate sample size, attrition
Pathak, 2013[57] United States Funding NR	Retrospective review	High	Retrospective, case series, inadequate sample size
Rubin, 2013[58] United States Health organization	Retrospective review	High	Retrospective, case series

Study Country Funding	Type of Study	Overall Risk of Bias Assessment	Rationale
van Nood, 2013[59] The Netherlands Government	Open-label randomized trial	High	Unblinded, inadequate sample size (n=43 randomized, n=13-17 per arm), stopped early
Brandt, 2012[107] United States No funding	Survey	High	Retrospective, survey design
Hamilton, 2012[60] United States Foundation, government	Case series	High	Case series, followup not reported
Jorup-Ronstrom, 2012[61] Sweden Funding NR	Observational	High	Retrospective, case series, inadequate sample size, outcomes not clearly defined
Kelly, 2012[62] United States Funding NR	Case series	High	Case series, inadequate sample size, adverse events not reported
Mattila, 2012[63] Finland Foundation	Retrospective review	High	Retrospective, case series
Mellow, 2011[64] United States Funding NR	Observational	High	Case series, inadequate sample size, selective CDI testing
Garborg, [65]2010[65] Norway Funding NR	Retrospective review	High	Retrospective, case series, heterogeneous sample (confirmed or suspected CDI), lack of systematic followup
Aas, 2003[66] United States Health organization	Retrospective review	High	Retrospective, case series, inadequate sample size, selective CDI testing
Previously identified studies			
Rohlke, 2010[67] United States No funding	Retrospective review	High	Retrospective, case series, inadequate sample size, population inclusion criteria (“recurrent CDI”) not defined, adverse events not reported
Yoon, 2010[68] United States No funding	Case series	High	Retrospective, case series, inadequate sample size
MacConnachie, 2009[69] United Kingdom Funding NR	Retrospective review	High	Retrospective, case series, inadequate sample size

Appendix Table F6. Probiotic adjunctive treatments study risk of bias

Study Country Funding	Overall Risk of Bias Assessment	Rationale
Newly identified randomized trials		
Ouwehand, 2014[70] China Industry	Moderate	Outcomes not clearly reported for CDI
Allen, 2013[71] United Kingdom Government	High	Underpowered for event rate, limited followup duration
Selinger, 2013[72] United Kingdom Industry, government	High	Underpowered for event rate, 45% did not complete study, trial stopped early due to low incidence of CDI
Pozzoni, 2012[73] Italy Hospital	High	Possible attrition bias, selective CDI testing, underpowered for event rate
Gao, 2010[74] China Industry	Moderate	Selective CDI testing.
Lonnermark, 2010[75] Sweden Funding NR	High	Possible attrition bias, selective CDI testing, limited followup duration, underpowered for event rate
Psaradellis, 2010[76] Canada Industry	High	Unclear randomization process and allocation concealment, possible attrition bias, selective CDI testing, underpowered for event rate, outcomes not reported by recurrence (heterogeneous population)
Safdar, 2008[77] United States Industry, NR	High	Underpowered for event rate
Beausoleil, 2007[78] Canada Industry	High	Unclear randomization process and allocation concealment, selective CDI testing, underpowered for event rate
Duman, 2005[79] Turkey Funding NR	High	Unclear randomization process and allocation concealment, open label, possible attrition bias, underpowered for event rate
Newly identified observational study		
Bakken, 2014[81] United States Case series None	High	No comparison group.
Maziade, 2013[80] Canada Open prospective Hospital	High	Observational design, unclear details of treatment/comparison groups
Previously identified trials		
Hickson, 2007[82] United Kingdom Foundation	High	Possible attrition bias, selective CDI testing, underpowered for event rate
Can, 2006[83] Turkey Funding NR	High	Unclear randomization process and allocation concealment, blinding patient or assessors; possible attrition bias, underpowered for event rate
Plummer, 2004[84] United Kingdom Funding NR	High	Unclear randomization process and allocation concealment, selective CDI testing, underpowered for event rate, outcomes not reported by carrier status (heterogeneous population)

Study Country Funding	Overall Risk of Bias Assessment	Rationale
Thomas, 2001[85] United States Industry	High	Possible attrition bias, selective CDI testing, CDI assessment by retrospective chart review, underpowered for event rate
Lewis, 1998[86] United Kingdom Health organization	High	Unclear randomization process and allocation concealment, unclear followup duration, underpowered for event rate
McFarland, 1995[87] United States	High	Unclear randomization process and allocation concealment, attrition bias, underpowered for event rate
Surawicz, 1989[88] United States Industry	High	Unclear allocation concealment, attrition bias, underpowered for event rate, outcomes not reported by carrier status (heterogeneous population)

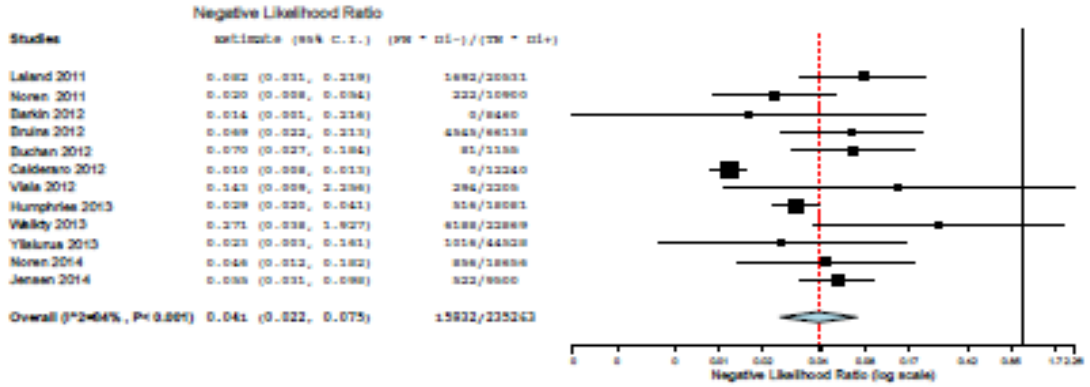
Appendix Table F7. Other adjunctive treatments study risk of bias

Study Country Funding	Type of Study	Overall Risk of Bias Assessment	Rationale
Newly identified studies			
Puri, 2015[89] United Kingdom None	Case Series	High	No comparator
Garey 2011[90] United States Industry	Randomized trial	High	Trial stopped early; unusually low cure rate for established comparator.
Laffan, 2011[91] United States Industry	Randomized trial	High	Inadequate sample size

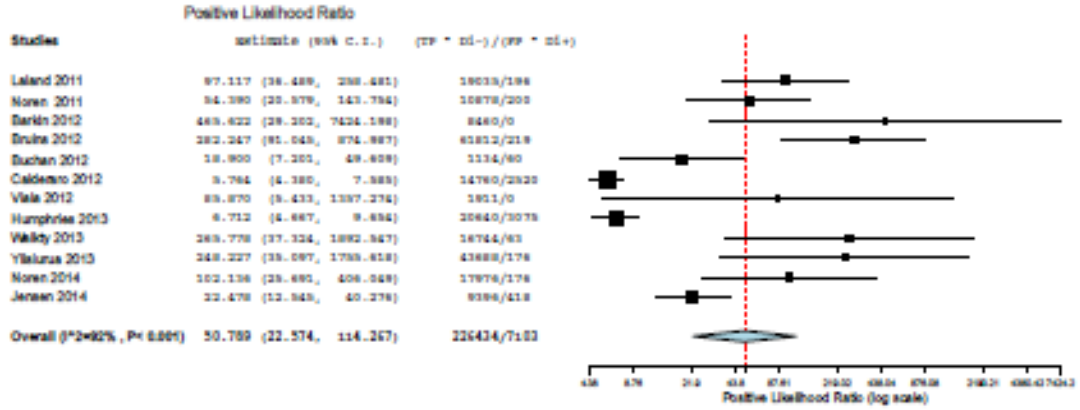
Appendix G. Detailed Analyses

KQ1 – Diagnostics

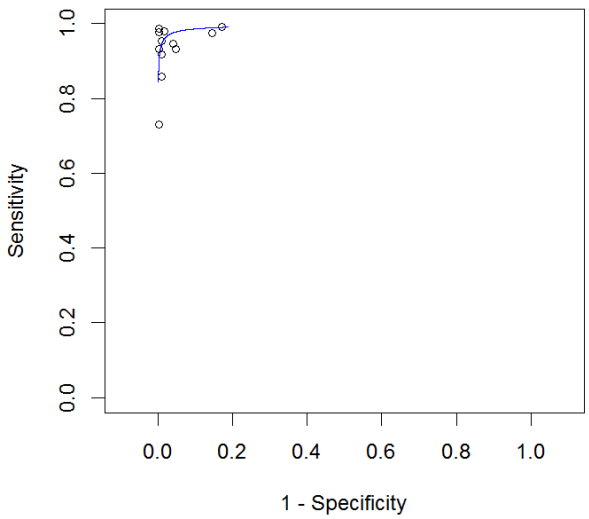
Appendix Figure G1. LAMP negative likelihood ratio



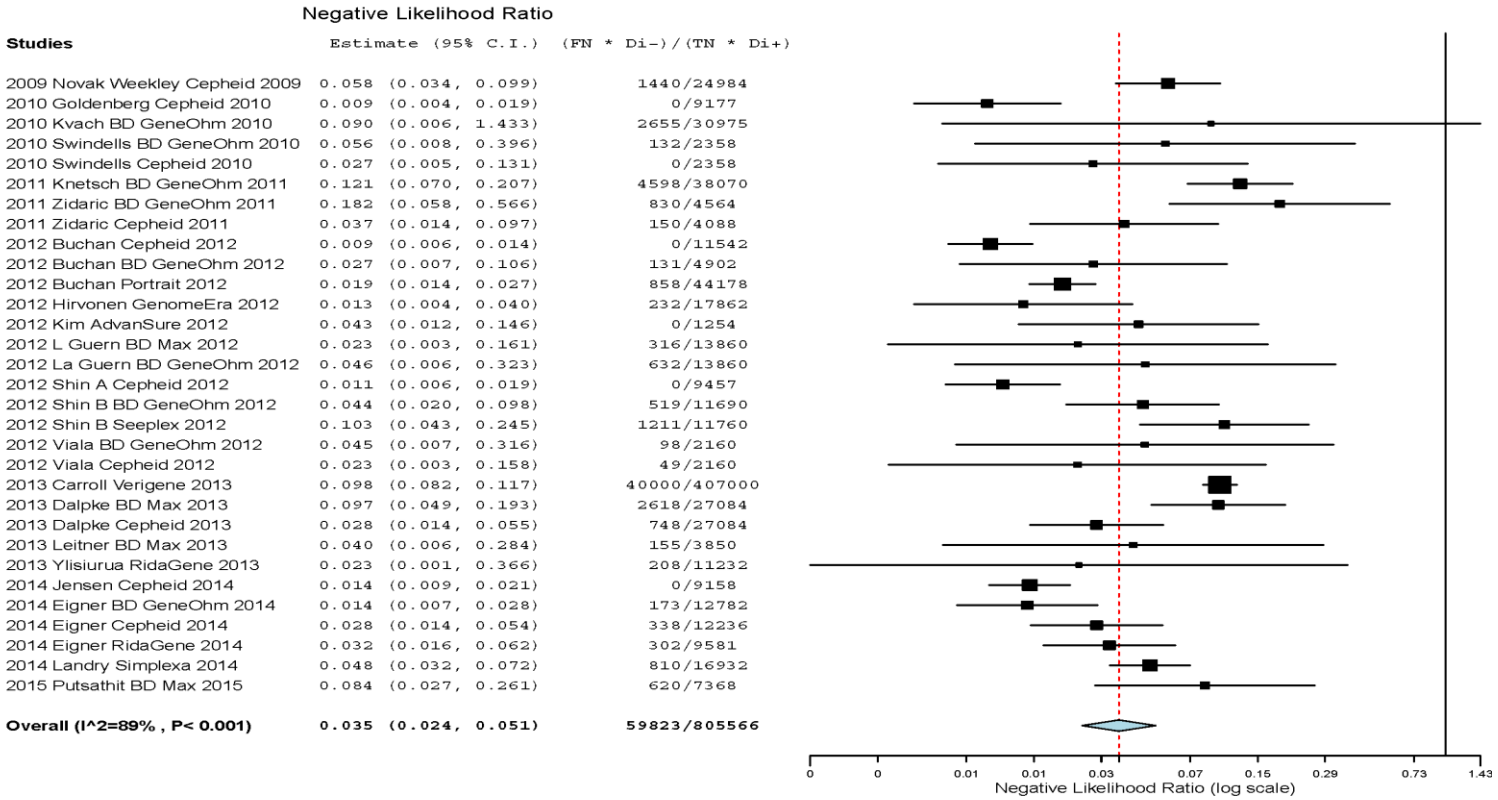
Appendix Figure G2. LAMP positive likelihood ratio



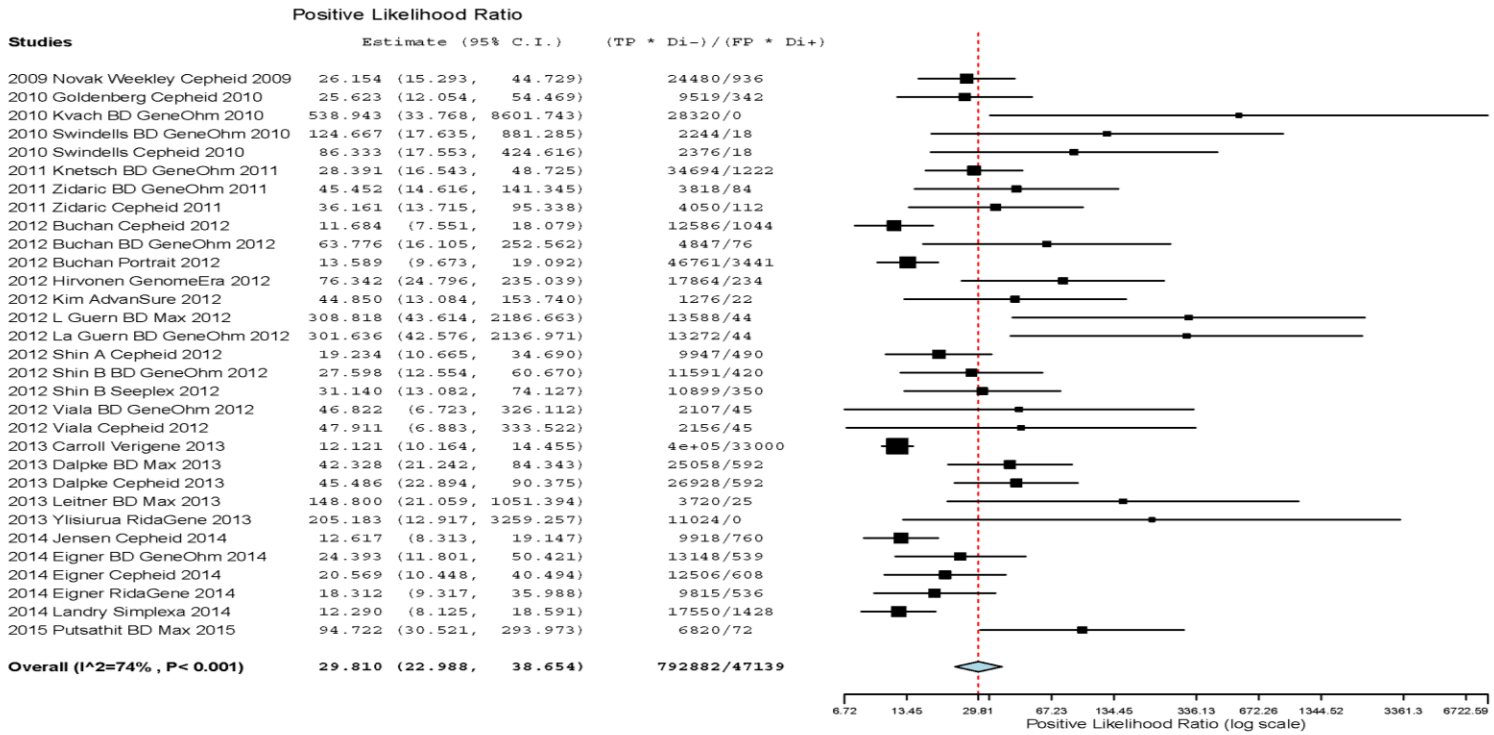
Appendix Figure G3. LAMP SROC



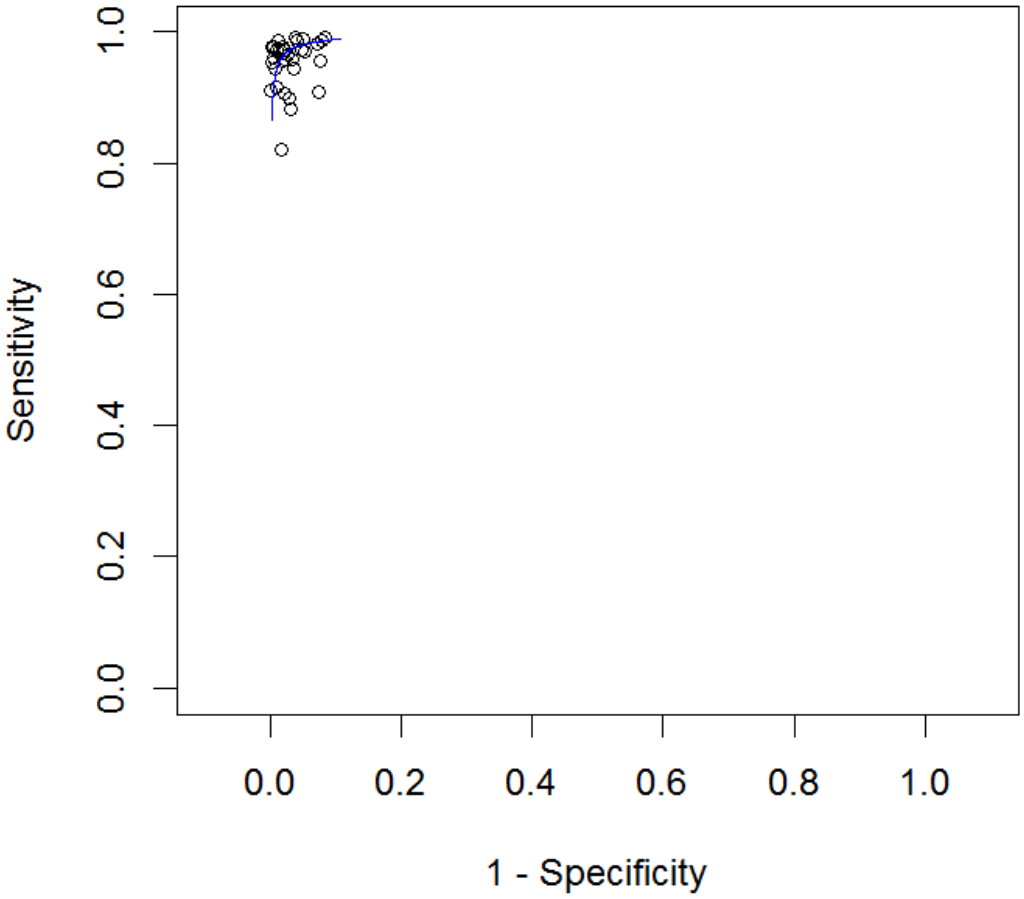
Appendix Figure G4. PCR negative likelihood tatio



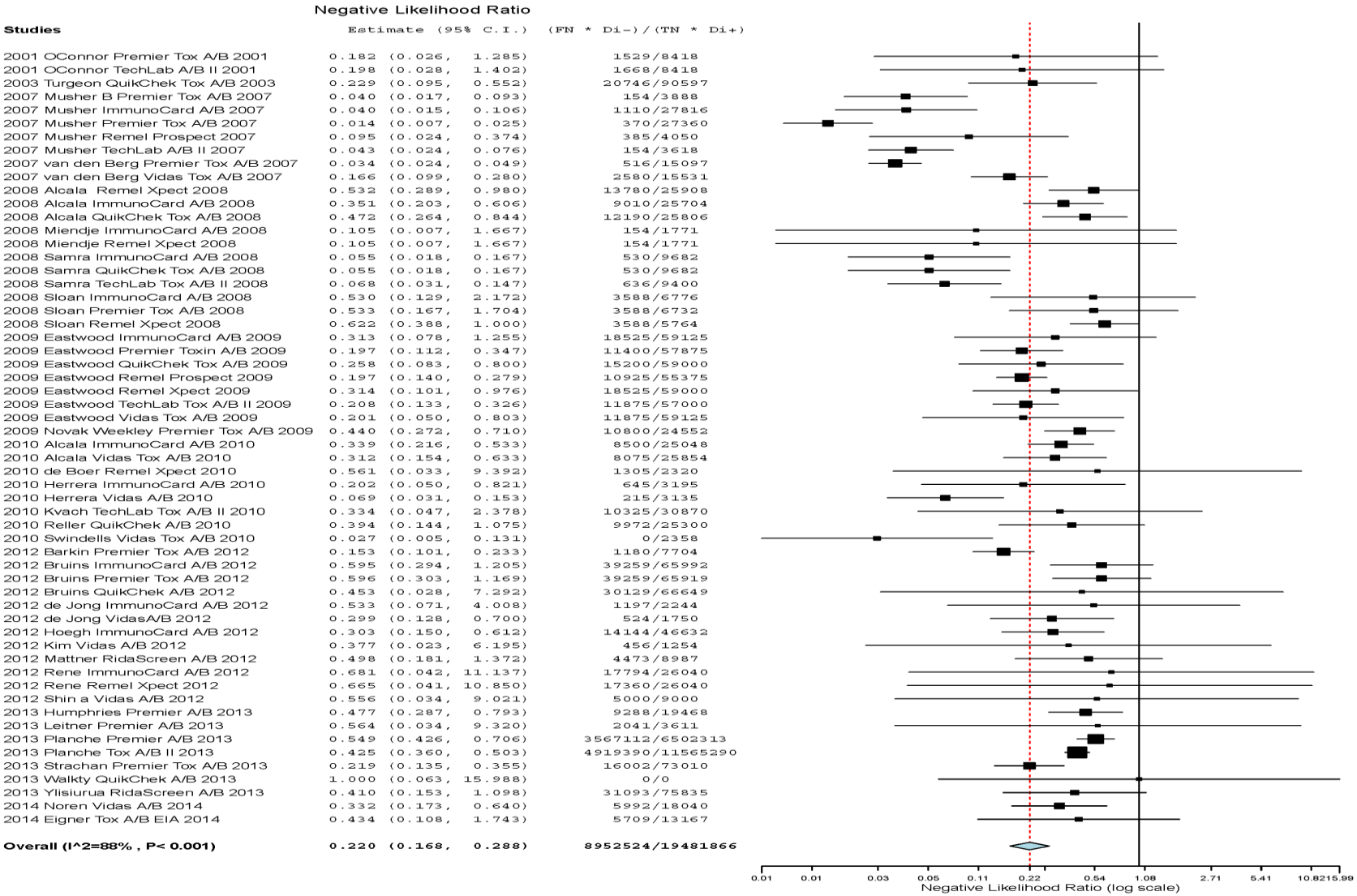
Appendix Figure G5. PCR positive likelihood ratio



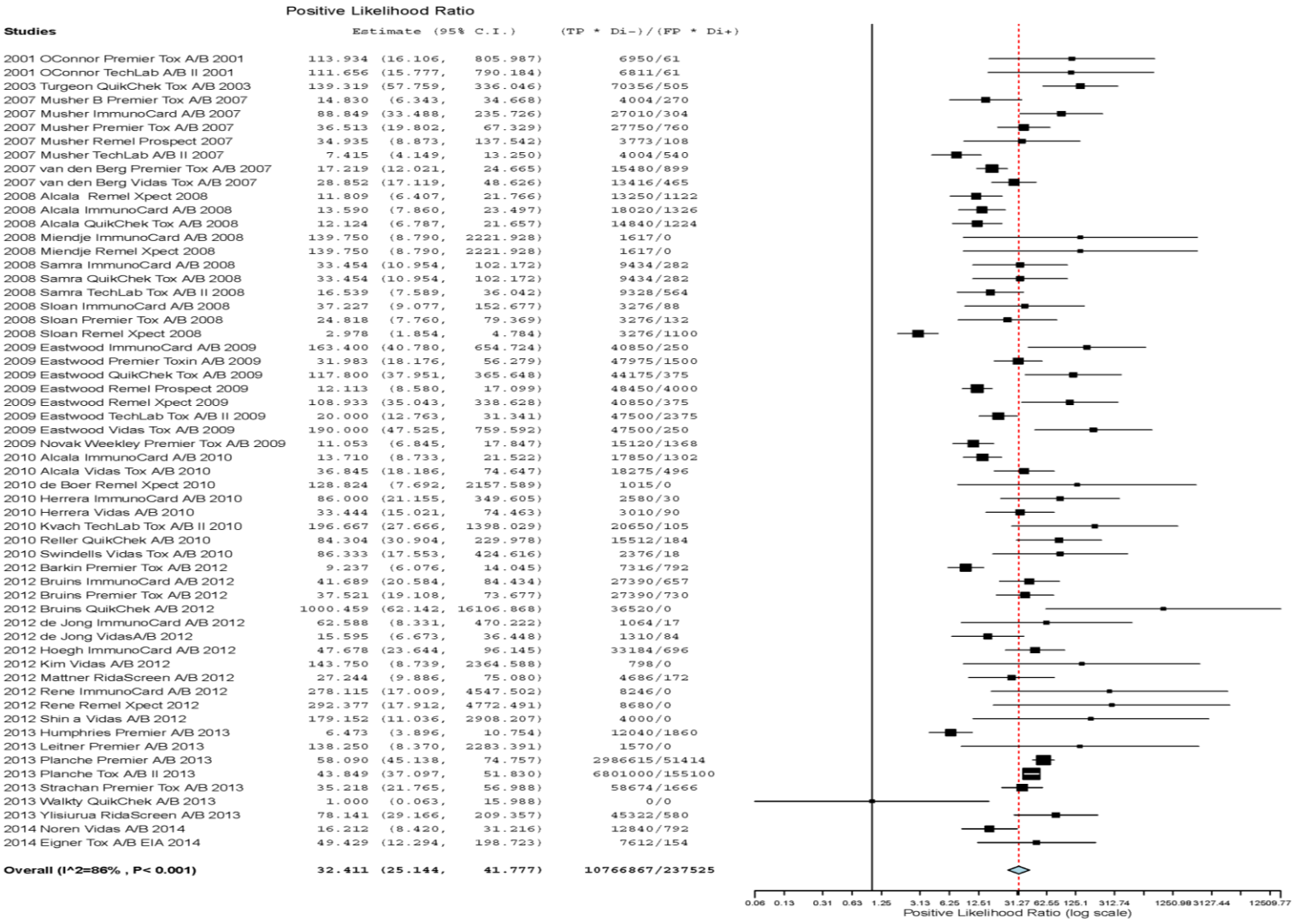
Appendix Figure G6. PCR SROC



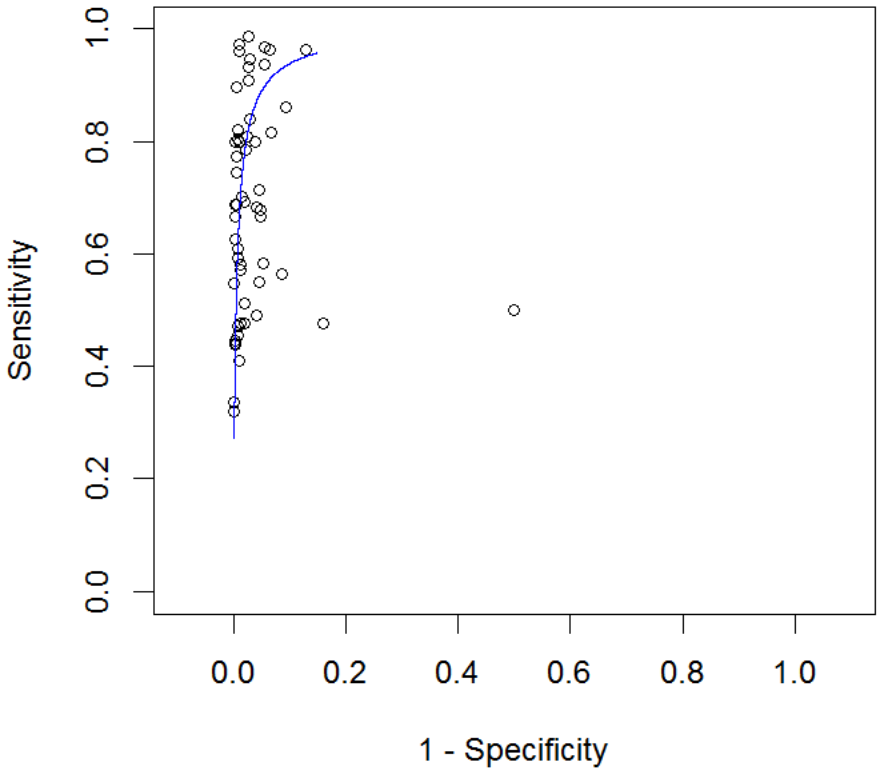
Appendix Figure G7. Toxin A/B negative likelihood ratio



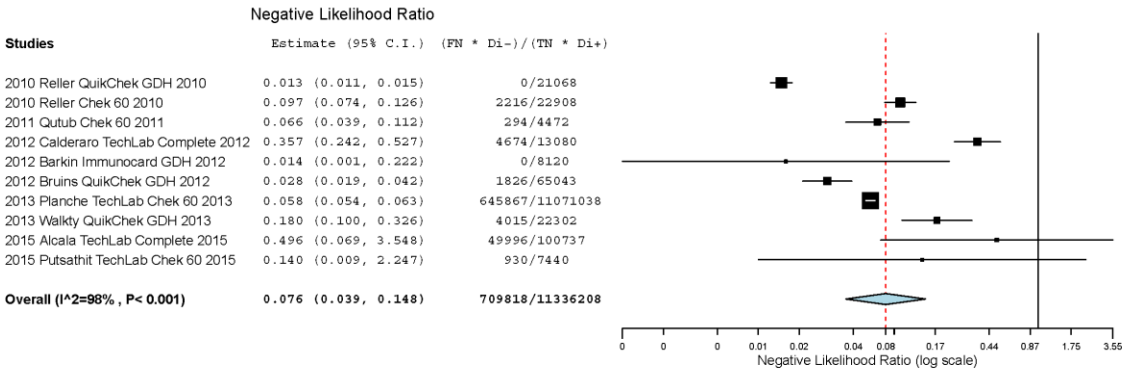
Appendix Figure G8. Toxin A/B positive likelihood ratio



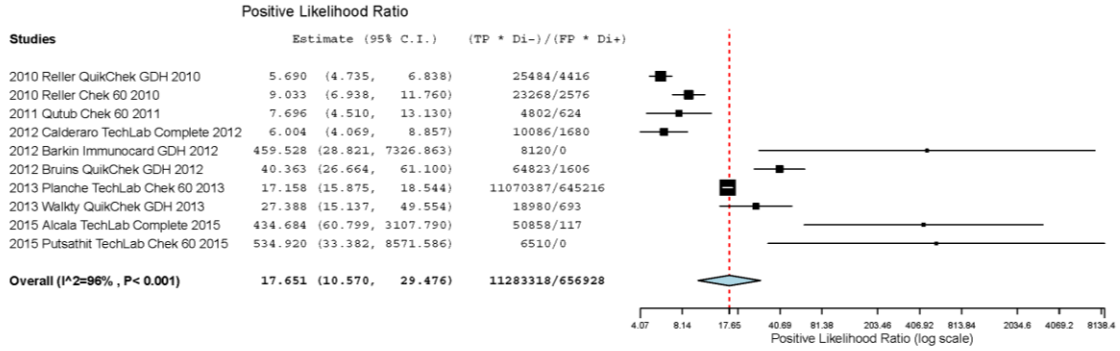
Appendix Figure G9. Toxin A/B SROC



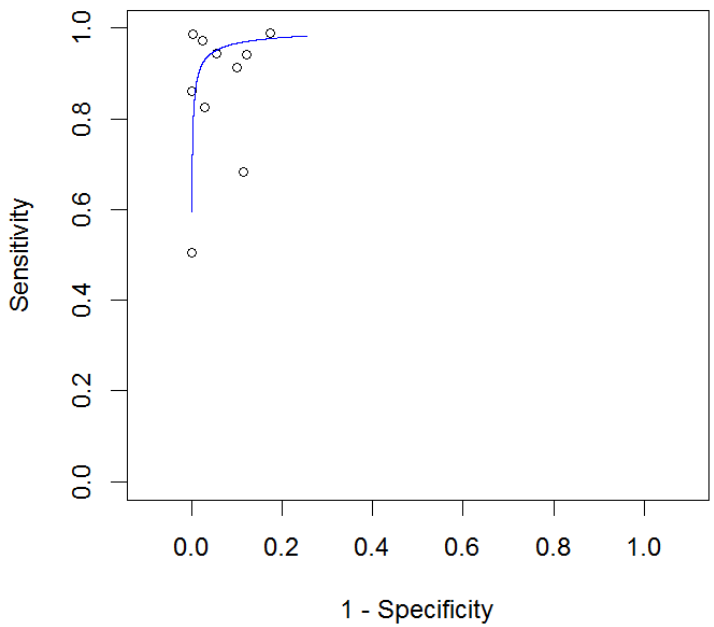
Appendix Figure G10. GDH negative likelihood ratio



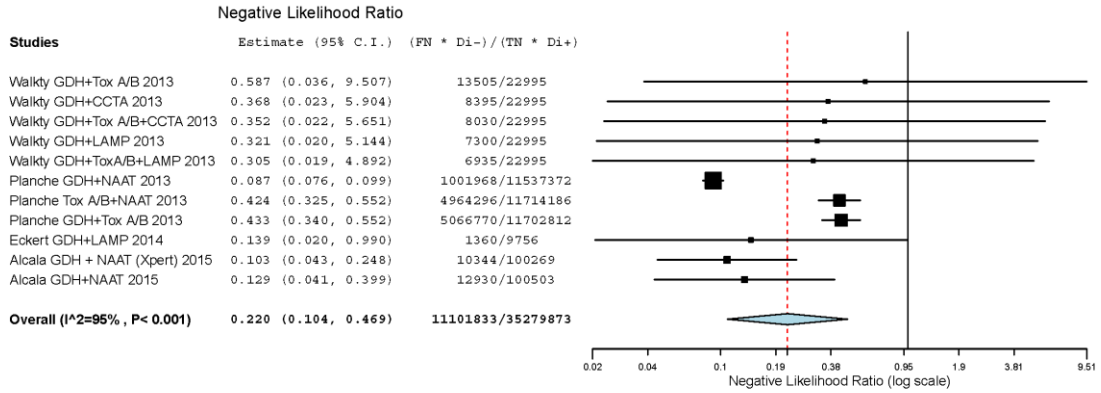
Appendix Figure G11. GDH positive likelihood ratio



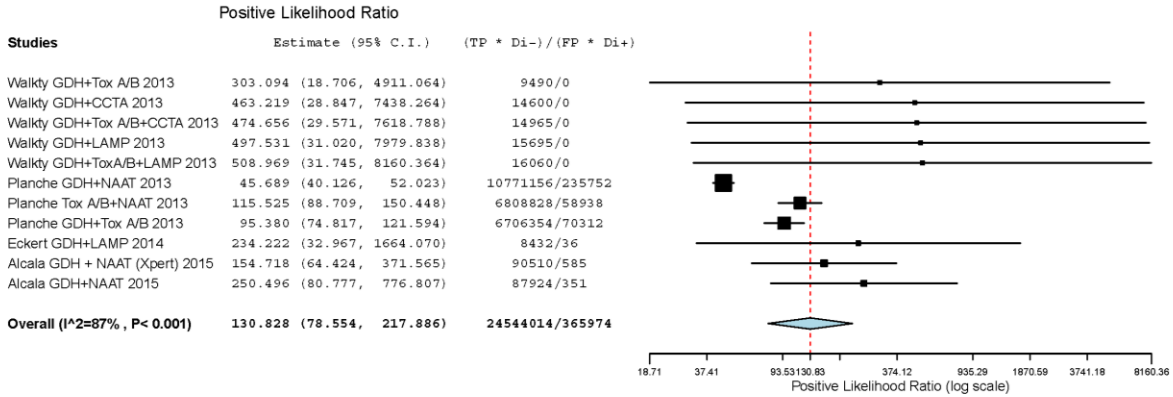
Appendix Figure G12. GDH SROC



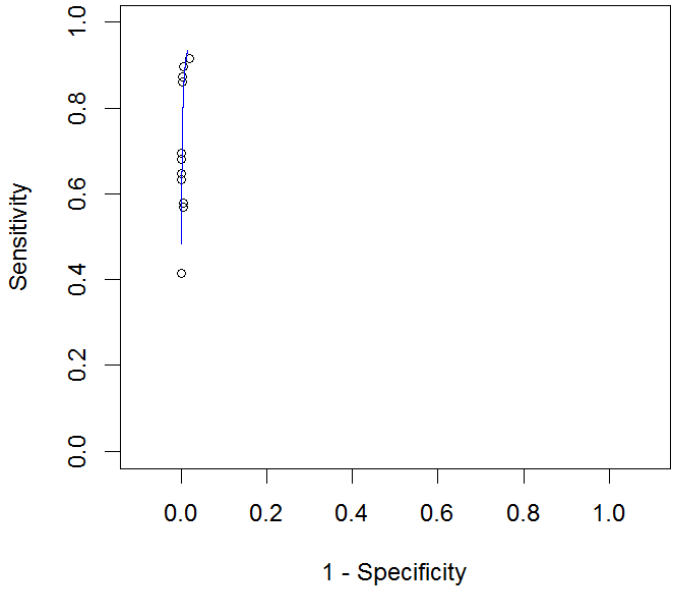
Appendix Figure G13. All test algorithms negative likelihood ratio



Appendix Figure G14. All test algorithms positive likelihood ratio



Appendix Figure G15. All test algorithms SROC



KQ2 – Prevention

Appendix Table G1. Prevention interventions, all with CDI incidence as outcome

Author, Year Country	Study Design	Population Setting	CDI Definition Timing Testing	Intervention	Study Findings
Antibiotic Stewardship					
Filice, 2013[92] United States	Systematic review 37 included studies 1 RCT, 5 interrupted time series (one of which overlapped with the original report) relevant to CDI incidence	Patients at risk for CDI Inpatient settings, not pediatric	Defined: based on individual study Timing: NA Testing: NA	Inpatient antimicrobial stewardship programs	Low strength evidence from 3 moderate and 3 high risk of bias studies that broad range of antimicrobial stewardship programs reduce CDI incidence (qualitative synthesis)
Transmission Interruption					
Rupp, 2012[93] United States	Quasi-experimental staged introduction trial in 3 cohorts, 19 months followed by 4 month wash-out	Patients at risk for CDI 689-bed academic medical center (not pediatric). Nebraska. Not outbreak setting.	Defined: CDC NHSN criteria Timing: Not reported Testing: Not reported	Chlorhexidine gluconate (CHG) bathing 3 days per week or daily	CDI RR 0.41 (95% CI, 0.29 to 0.59) for daily bathing, 0.71 (95%, CI 0.57 to 0.89) for 3 times per week, and 1.85 (95% CI, 1.38 to 2.53) for CDI in washout period compared with daily bathing. Daily more effective than 3 times per week Adverse Events: no events reported
Noto, 2015[94] United States	Pragmatic cluster randomized cross-over trial, 1 year	Patients at risk for CDI 5 adult intensive care units, tertiary medical center, Nashville Tennessee. Not outbreak setting	Defined: CDC NHSN criteria Timing: Not reported Testing: Not reported	Chlorhexidine gluconate bathing daily for 10-week period followed by 2-week wash-out. Daily bathing assigned 3 times over the study	CDI rate not different between groups by both intention-to-treat and as-treated analyses.
Alfa, 2015[95] Canada	Prospective interrupted time series, 3 years historical, 1 year intervention	Patients at risk for CDI 538-bed tertiary care hostpital (medicine, cardiac, surgery, women and child wards), Manitoba. Not outbreak setting.	Defined: Not reported Timing: Not reported Testing: 4 step algorithm	Daily disinfectant cleaning with hydrogen peroxide disposable wipes on high-touch surfaces in patient care rooms	When compliance was >80%, CDI dropped from 54 to 39 cases/10,000 patients (p=.0005)

Author, Year Country	Study Design	Population Setting	CDI Definition Timing Testing	Intervention	Study Findings
Manian, 2013[97.1.90] United States	Retrospective Pre/post single site. 1 year followup.	Patients at risk for CDI (not pediatric or rehabilitation) 900-bed teaching hospital, St. Louis, MO. Not outbreak setting	Defined: diarrhea with positive test for toxin A/B Timing:3 days after admission or 7 days after discharge Test: EIA (Meridian)	Hydrogen peroxide vapor in sealed room	CDI incidence rate dropped from 0.88/1000 patient days to 0.55/1000 patient days (0.63. 95% CI, 0.50 to 0.79) Adverse Events: Reported no events related to cleaning
Passaretti, 2013[98] United States	Prospective cohort intervention in 3 cohorts. 1 year, 6 month followup	Patients at risk for CDI 994-bed tertiary hospital. Maryland. Not outbreak setting.	Defined: Not reported Timing: 48 hours after admission Test: Not reported	Hydrogen peroxide vapor in sealed room	Trend in reduced rate but no statistical difference in CDI incidence rate. Adverse Events: Reported no events related to cleaning
Levin, 2013[96] United States	Pre/post single site. 1 year followup	Patients at risk for CDI 140-bed community hospital, Western MA. Not outbreak setting.	Defined: CDC NHSN criteria Timing: not reported Test: PCR and Immunocard Toxins A and B	Portable pulsed xenon ultraviolet light used in 3 7-minute sessions per patient room. Device operated remotely by cleaning personel. Safety feature turns off light if door opens.	CDI rates declined from 9.46 per 10,000 in 2010 to 4.45 per 10,000 in 2011, a 53% reduction. Declines also in deaths, from 6 to 1, and coloctomies, from 3 to 0.
Stone, 2012[99] United Kingdom	Prospective, ecological, interrupted time series. 3 year followup after roll-out	Patients 65+ years at risk for CDI 187 hospital trusts in England . Not outbreak setting.	Defined: Not reported Timing: 48 hours after admission Test: Not reported	Clean your hands campaign: alcohol rub at bedside, reminder posters, compliance audit and feedback, materials to patients empowering them to remind healthcare workers to clean their hands	CDI fell from peak of 16.75 to 9.49 cases per 10,000 bed days. Soap use independently associated with reduced CDI. CDI was not associated with alcohol gel in multivariate analysis.
DiDiodato, 2013[100] Canada	Prospective, ecological, interrupted time series. 3 years	Patients at risk for CDI 166 acute care hospitals, Ontario. Not outbreak setting.	Defined: Not reported Timing: 72 hours after admission Test: Not reported	Ontario Just Clean Your Hands patient safety initiative. Education and training program. Mandated hand hygiene audits and public reporting	No statistical differences found
Bearman, 2010[101] United States	Prospective Pre/post single site. 6 month followup	Patients at risk for CDI 18 bed surgical intensive care unit (820-bed academic medical center) Virginia. Not outbreak setting.	Defined: Not reported Timing: Not reported Test: Not reported	Universal gloving with emollient-impregnated gloves	No significant differences in CDI incidence

Author, Year Country	Study Design	Population Setting	CDI Definition Timing Testing	Intervention	Study Findings
Multicomponent					
Brakovich, 2013[102] United States	Prospective Pre/post single site design. 2 year followup	Patients at risk for CDI 50-bed long-term acute care hospital, southeastern United States. Not outbreak setting.	Defined: unclear Timing: first event at least 3 days after admission Test: for antigen marker, *C. diff* glutamate dehydrogenase, toxins A and B	Tiered approach: Cleaning education plan developed based on empiric test of site terminal cleaning Microfiber mops Hydrogen peroxide vapor equipment/services Bleach Contact isolation Hand hygiene Antimicrobial stewardship plan Quarterly feedback	CDI incidence rate: 44.25% decrease in cumulative rate, sustained over 2 years. (Cumulative rate drop from 56.52 to 31.51)
Bishop, 2013[103] United States	Prospective pre/post single site design. 3 year followup	Surgical patients at risk for CDI Connecticut community hospital (Stamford Hospital). Not outbreak setting	Defined: CDC NHSN criteria Timing: within 30 days of hospital exposure Test: EIA (2007-2008) PCR (2009-2010)	Resident rounding protocol Antibiotic stewardship Restriction of gastric acid suppression Contact isolation Hand hygiene (Terminal cleaning previously introduced)	CDI incidence rate: 41% decrease in annual rate, sustained over 3 year 64% decrease in patient days. (2.8/1000 vs 1.8/1000
Mermel, 2013[104] United States	Time series single site design. 6 year, 9 month followup. Prospective monitoring	Patients at risk for CDI 719-bed Rhode Island tertiary care hospital (Rhode Island Hospital). Post-outbreak setting.	Defined: CDC NHSN criteria Timing: includes patients with 30 day readmit with diarrhea and confirmed toxin present. Test: PCR	Progressive roll-out of elements of CDI control plan based on risk assessment Monitor CDI morbidity/ mortality Improve testing using PCR Enhance environmental cleaning CDI treatment plans Other interventions	CDI incidence rate: drop from 12.2/1000 to 3.6/1000. Annual mortality drop from 52 to 19
Price, 2010[105] United Kingdom	Interrupted time series single site design; 12 months pre, 15 months post. Retrospective	Patients at risk for CDI 820-bed teaching hospital and tertiary services (Brighton and Sussex University Hospital NHS Trust). Not outbreak setting	Defined: Liquid stool and positive test for Toxins A or B Timing: More than 3 days after admission or before 3 days after discharge Test: Not reported	Restrictive antibiotic use and isolation or cohorting active cases	Increase in the CDI reduction rate from 3% to 8% per month.

Author, Year Country	Study Design	Population Setting	CDI Definition Timing Testing	Intervention	Study Findings
You, 2014[106] Korea	Retrospective Pre/post single site design, 9 month followup	Patients at risk for CDI Medical intensive care unit (Korea). Not outbreak setting.	Defined:symptoms and positive test Timing: Not reported Test: PCR	Education, isolation, hand hygiene, contact precautions, and environmental disinfection. Did not include restricting antimicrobial agents	CDI incidence rate: decrease from 4.7/1000 to 1.53/1000 patient days. Overall hospital rate increased over same period.

CDI=*C. difficile* infection; CDC NHSF-Centers for Disease Control and Prevention National Healthcare Safety Network; SHEA=Society for Healthcare Epidemiology of America

KQ3 – Standard Treatment

Initial Cure

A single new RCT comparing metronidazole, vancomycin, and tolevamer was published in 2014.[37] Tolevamer was inferior to both metronidazole and vancomycin, and is not discussed further since it is not licensed by the U.S. Food and Drug Administration. The results of the metronidazole and vancomycin arms (n = 537) showed that vancomycin led to a significant increase in subjects achieving initial cure (81.1 percent versus 72.7 percent; P = .02). When combined with the three previous RCTs comparing metronidazole to vancomycin,[41-43] the percentage of subjects achieving initial cure was significantly higher among those receiving vancomycin (83.9 percent versus 75.7 percent; RR 1.08, 95% CI 1.02 – 1.15).

The second RCT identified in our update is a trial of fidaxomicin versus vancomycin (n = 509).[38] This study is the second of two studies that led to the approval of fidaxomicin for the treatment of CDI in the United States. Consistent with the first study, which was included in our original review, fidaxomicin performed similarly to vancomycin for the outcome of initial cure. Specifically, the percentage of subjects meeting initial cure did not differ significantly by treatment received (87.7 percent for fidaxomicin versus 86.8 percent for vancomycin; P = .79). Combining these results with those from the first study of fidaxomicin versus vancomycin[40] led to a similar finding of no significant difference in initial cure when stratified by treatment received (87.6 percent versus 85.6 percent; RR 1.02, 95% CI 0.98 – 1.07).

Recurrent CDI

The newly identified trial of metronidazole versus vancomycin[37] demonstrated no difference between the two agents for the outcome of recurrent CDI (20.6 percent versus 23.0 percent; P = .64). Similarly, when data from this study were pooled with the three previous RCTs comparing metronidazole versus vancomycin, no significant differences were observed (16.5 percent versus 18.7 percent; RR 0.89, 95% CI 0.65 – 1.23).

In contrast, the trial of fidaxomicin versus vancomycin demonstrated that use of fidaxomicin led to significantly fewer subjects having recurrent CDI (12.7 percent versus 26.9 percent; P = .002). Similarly, when pooled with the data from the prior study of fidaxomicin and vancomycin,[40] recurrence remained less likely after fidaxomicin treatment (14.1 percent versus 26.1 percent; RR 0.55, 95% CI 0.42 – 0.71).

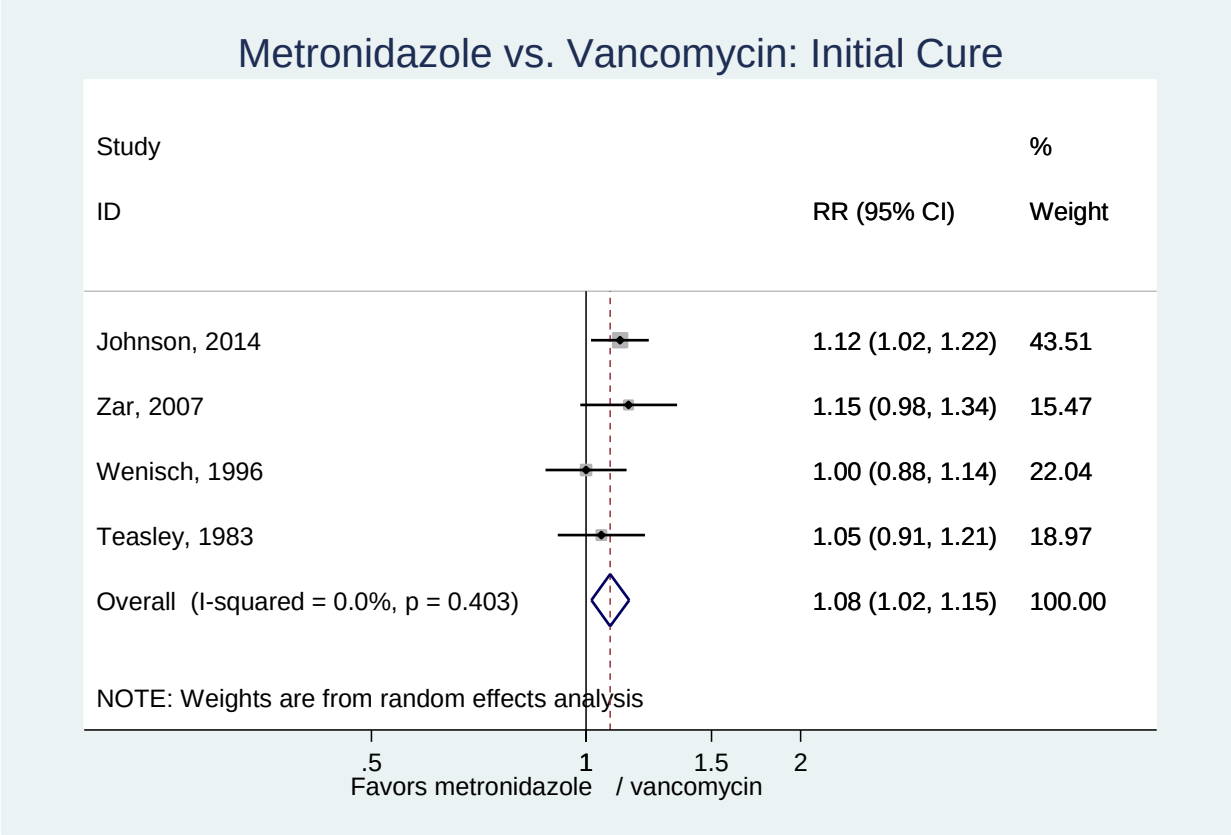
Finally, the observational study noted similar recurrence rates after oral metronidazole and vancomycin (20.6 percent and 19.0 percent, respectively), but higher rates after intravenous metronidazole (50.0 percent; P = .007).

Appendix Table G2. Initial clinical cure: # subjects / # randomized (%) for vancomycin versus metronidazole

Study	Vancomycin	Metronidazole	RR [95% CI]
Johnson, 2014[37]	210/259 (81)	202/278 (73)	1.12 [1.02 to 1.22]
Zar, 2007[41]	69/82 (84)	66/90 (73)	1.15 [0.98 to 1.34]
Wenisch, 1996[42]	29/31 (94)	29/31 (94)	1.00 [0.88 to 1.14]
Teasley, 1983[43]	51/56 (91)	39/45 (87)	1.05 [0.91 to 1.21]
Totals	359/428 (84)	336/444 (76)	1.08 1.01 to 1.15]]

CI = confidence interval; RR = relative risk

Appendix Figure G16. Initial clinical cure: vancomycin versus metronidazole

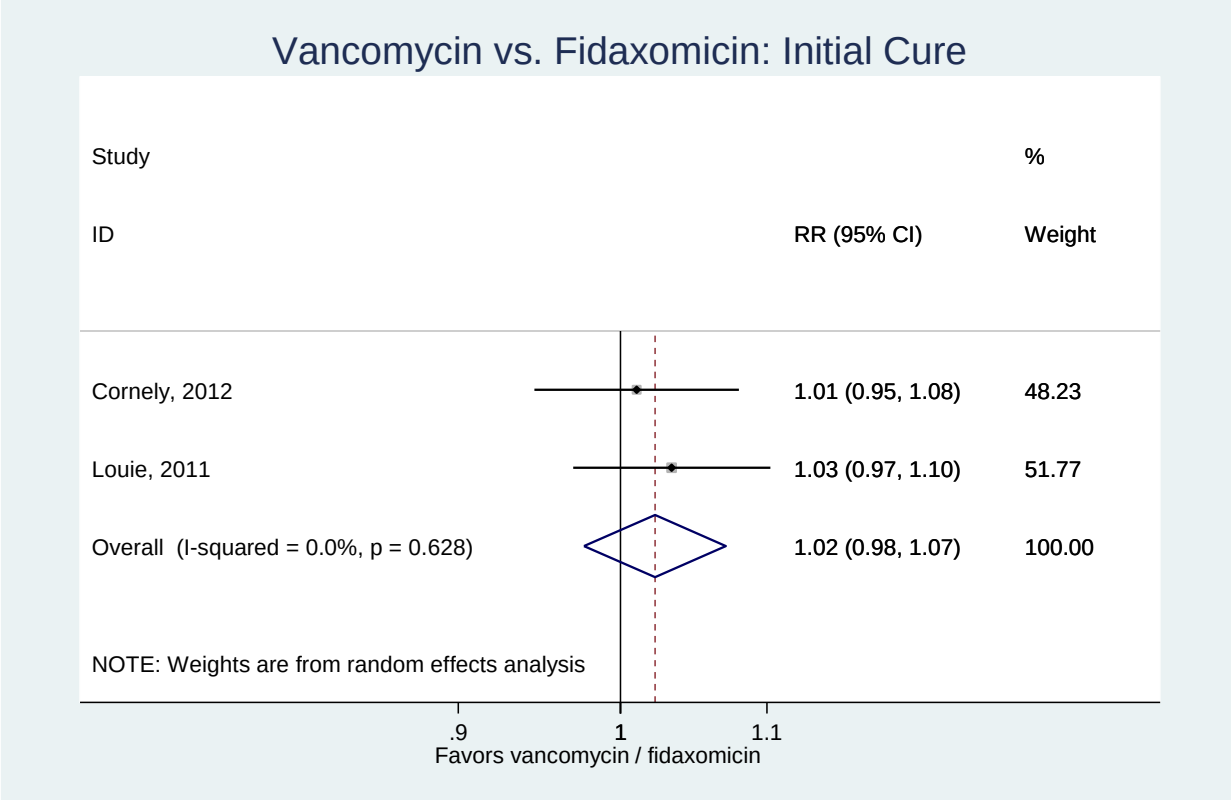


Appendix Table G3. Initial clinical cure: # subjects / # randomized (%) for fidaxomicin versus vancomycin

Study	Fidaxomicin	Vancomycin	RR [95% CI]
Cornely, 2012[38]	221/252 (88)	223/257 (87)	1.01 [0.95 to 1.08]
Louie, 2011[40]	253/289 (88)	265/313 (85)	1.03 [0.97 to 1.10]
Totals	474/541 (88)	488/570 (86)	1.02 [0.98 to 1.07]

CI = confidence interval; RR = relative risk

Appendix Figure G17. Initial clinical cure: vancomycin versus fidaxomicin

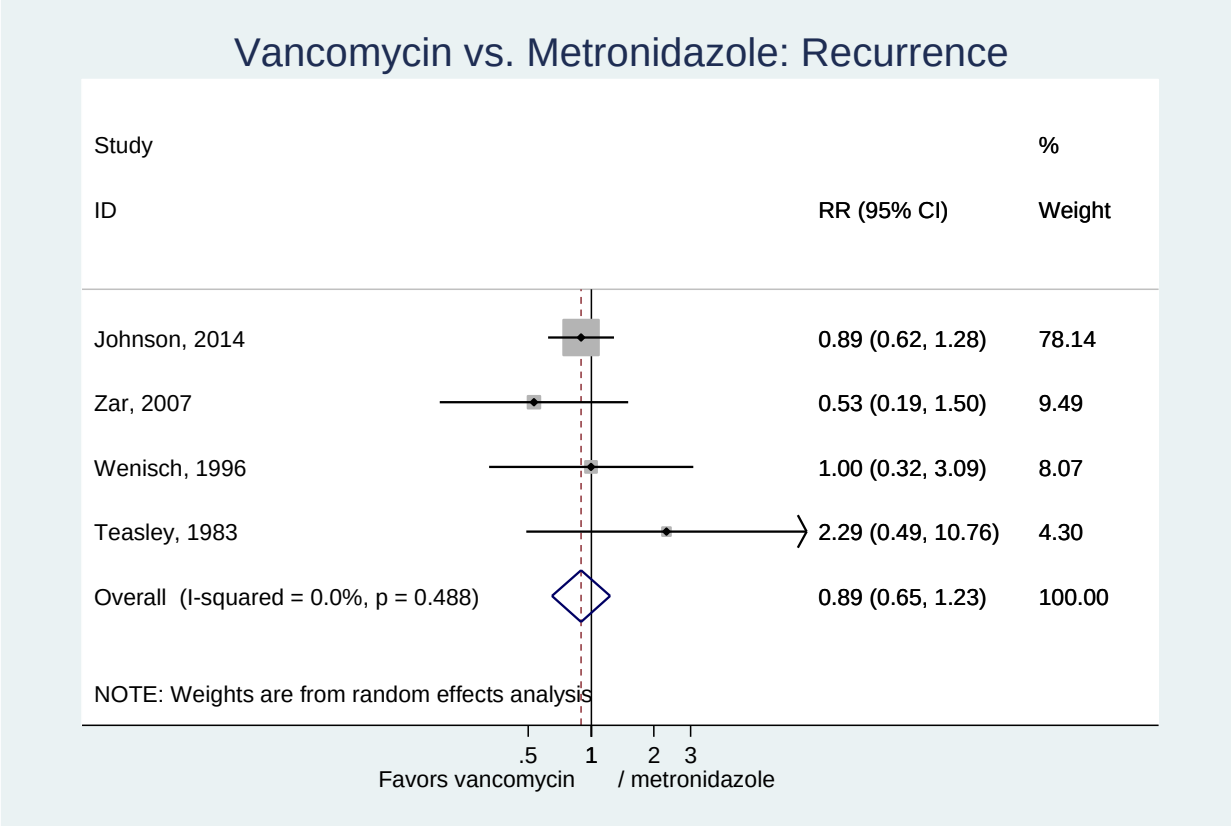


Appendix Table G4. Clinical recurrence: # subjects / # initially cured (%) for vancomycin versus metronidazole

Study	Vancomycin	Metronidazole	Relative Risk [95% CI]
Johnson, 2014[37]	43/209 (21)	49/213 (23)	0.89 [0.62 to 1.28]
Zar, 2007[41]	5/69 (7)	9/66 (14)	0.53 [0.19 to 1.50]
Wenisch, 1996[42]	5/29 (17)	5/29 (17)	1.00 [0.32 to 3.09]
Teasley, 1983[43]	6/51 (12)	2/39 (5)	2.29 [0.49 to 10.76]
Totals	59/358 (16)	65/347 (19)	0.89 [0.65 to 1.23]

CI = confidence interval

Appendix Figure G18. Recurrence of CDI: metronidazole versus vancomycin

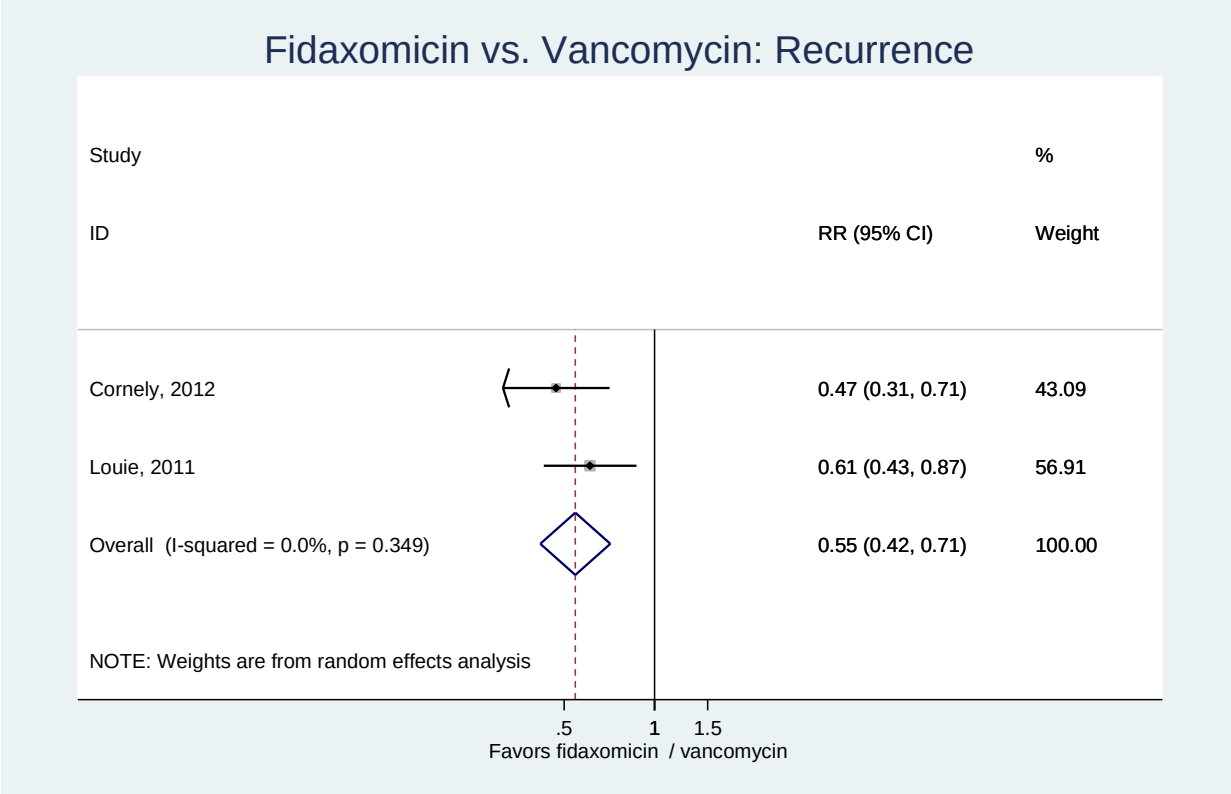


Appendix Table G5. Clinical recurrence: # subjects / # initially cured (%) for fidaxomicin versus vancomycin

Study	Fidaxomicin	Vancomycin	Relative Risk [95% CI]
Cornely, 2012[38]	28/221 (13)	60/223 (27)	0.47 [0.31 to 0.71]
Louie, 2011[40]	39/253 (15)	67/265 (25)	0.61 [0.43 to 0.87]
Totals	67/474 (14)	127/488 (26)	0.55 [0.42 to 0.71]

CI = confidence interval

Appendix Figure G19. Recurrence of CDI: vancomycin versus fidaxomicin



Appendix Table G6. Severe disease: # subjects / # (%)

Study	Fidaxomicin	Vancomycin	Metronidazole	Finding
Cornely, 2012[38] initial cure	48/63 (76)	43/61 (71)		RR 0.81 [CI 0.45-1.45]
Cornely, 2012[38] recurrence	4/48 (8)	14/43 (33)		RR 0.26 [CI 0.09-0.72] results fragile to missing or reassignment
Johnson, 2014[37] Initial cure		50/64 (79)	61/92 (66)	RR 0.65 [CI 0.38-1.12]
Zar 2007[41] Initial cure		24/31 (78)	25/38 (66)	RR 1.20 [CI 0.92-1.57]

KQ4 – Nonstandard Treatment

FMT for Recurrent CDI

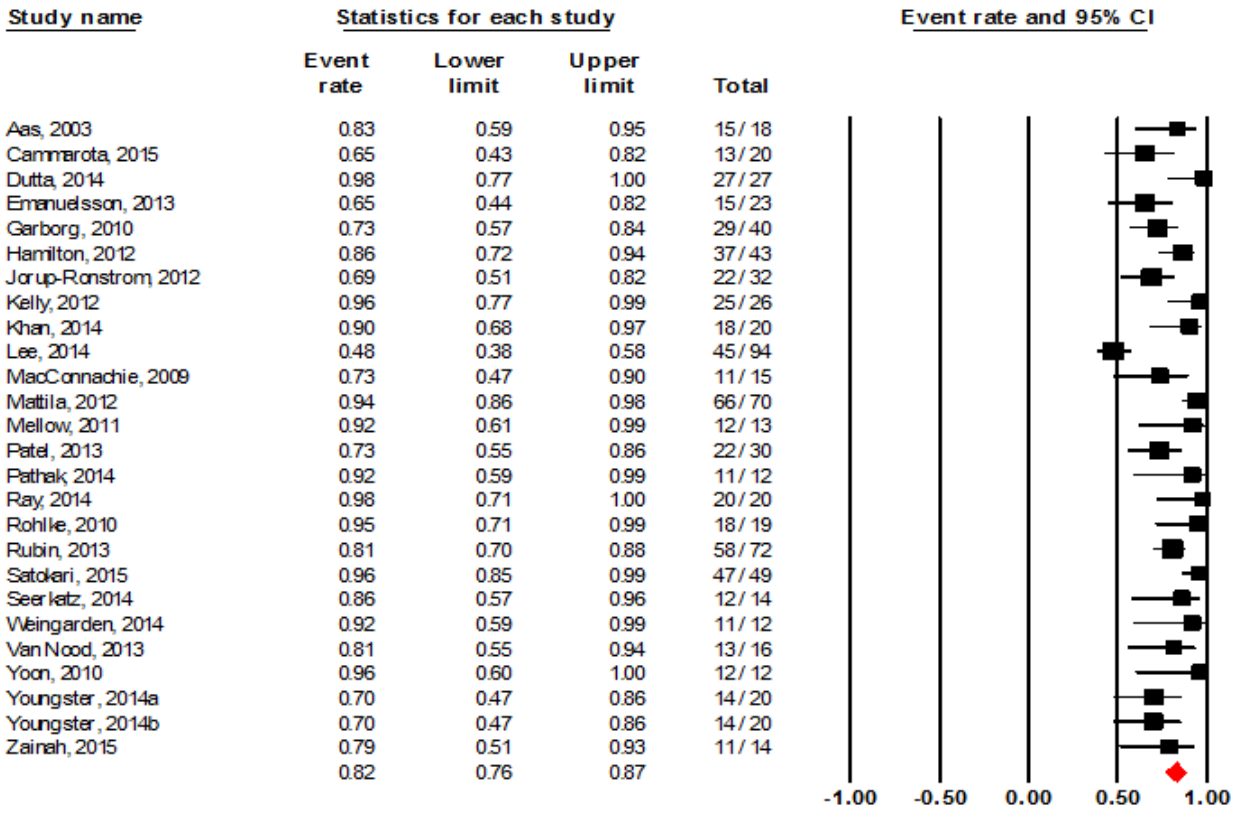
We identified 26 studies that addressed FMT for recurrent CDI of which three were small size RCTs and the remaining were observational. We identified two studies that included both recurrent and active CDI.[57,68] The studies included individuals between the ages of 7 and 90 years, with children included in one study. In 18 of the 21 studies, >55 percent of the participants were women. Two studies reported race and ethnicity distribution.[47,62] One of these studies enrolled 21 indivduals for FMT of which 74 percent were white, 22 percent black ,and 4 percent Asian.[47] The other study enrolled 26 individuals, 100 percent of whom were white.[62] Most studies were small, enrolling 12 to 94 individuals. Followup was variable, and ranged from 3 weeks to 8 years. Outcomes reported were resolution of diarrhea or symptoms, recurrence. and adverse events.

The three RCTs are noteworthy. One unblinded, three-arm RCT, conducted in the Netherlands, enrolled 43 adults with recurrent CDI with mean age of 70, 43 percent women.[59] Patients were randomized to oral vancomycin, FMT, or vancomycin plus bowel lavage. Followup was 10 weeks and the endpoint was resolution of diarrhea. The study was stopped early due to a large difference in the FMT and comparator groups (81 percent versus 31 percent and 23 percent). FMT was administered via nasoduodenal tube. However, the CDI rate in the comparator groups was unusually low.

Cammarota and colleagues conducted an additional unblinded trial of FMT via colonoscopy versus a vancomycin regimen that was given for at least 3 weeks, with the latter half given in a pulsed fashion (dosed every 2-3 days).[44] In patients with pseudomembranous colitis, the FMT protocol was amended after two patients to give FMT infusions every 3 days until resolution of colitis, versus the single infusion given to patients with CDI without pseudomembranous colitis. This study enrolled 39 subjects, with a mean age of 73. The primary endpoint was resolution of diarrhea associated with CDI at 10 weeks after the end of treatment. When analyzed by resolution after a single course of treatment (FMT or vancomycin), 65 percent of subjects had resolution of diarrhea with FMT, versus 26 percent with vancomycin. The authors noted that administering multiple courses of FMT increased the success rate to 90 percent in the FMT group and that multiple antibiotic courses increased the success rate to 53 percent in the vancomycin group. This study was also stopped early after an interim analysis.

Youngster and colleagues conducted an unblinded RCT that randomized 20 individuals with recurrent CDI, with mean age of 54, to colonoscopic or nasogastric administration of FMT.[54] The study endpoint was resolution of diarrhea without relapse within 8 weeks. The authors found no difference between the two modalities of FMT administration.

Appendix Figure G20. Resolution of symptoms after initial FMT for recurrent CDI, all routes



Appendix Table G7. Resolution of symptoms after initial FMT for recurrent CDI

Study	Events / Sample Size (Event Rate)	95% CI Lower Limit	95% CI Upper Limit
Aas, 2003[66]	15/18 (83)	0.59	0.95
Cammarota, 2015[44]	13/20 (65)	0.43	0.82
Dutta, 2014[47]	27/27 (98)*	0.77	1.0
Emanuelsson, 2013[55]	15/23 (65)	0.44	0.82
Garborg, 2010[65]	29/40 (73)	0.57	0.84
Hamilton, 2012[60]	37/43 (86)	0.72	0.94
Jorup-Ronstrom, 2012[61]	22/32 (69)	0.51	0.82
Kelly, 2012[62]	25/26 (96)	0.77	0.99
Khan, 2014[48]	18/20 (90)	0.68	0.97
Lee, 2014[49]	45/94 (48)	0.38	0.58
MacConnachie, 2009[69]	11/15 (73)	0.47	0.90
Mattila, 2012[63]	66/70 (94)	0.86	0.98
Mellow, 2011[64]	12/12 (92)	0.61	0.99
Patel, 2013[56]	22/30 (73)	0.55	0.86
Pathak, 2014[57]	11/12 (92)	0.59	0.99
Ray, 2014[50]	20/20 (100)	0.71	1.00
Rohlke, 2010[67]	18/19 (95)	0.71	0.99
Rubin, 2013[58]	58/72 (81)	0.70	0.88
Satokari, 2015[45]	47/49 (96)	0.85	0.99
Seekatz, 2014[51]	12/14 (86)	0.57	0.96
Van Nood, 2013[59]	13/16 (81)	0.55	0.94
Weingarden, 2014[52]	11/12 (92)	0.59	0.99
Yoon, 2010[68]	12/12 (96)*	0.60	1.0
Youngster, 2014a[53]	14/20 (70)	0.47	0.86
Youngster, 2014b[54]	14/20 (70)	0.47	0.86
Zainah, 2015[46]	11/14 (79)	0.51	0.93
Total	518/612 (85)	0.76	0.87

CI=confidence interval

FMT for Refractory CDI

Three studies reported outcomes for FMT in individuals with refractory CDI (defined as an episode that did not respond to antibiotic treatment). All were from case series, totaling 19 individuals.[46,53,64] Overall, there was insufficient strength of evidence supporting the role of FMT in refractory CDI. Unfortunately, few FMT studies provided detailed patient information to identify whether included patients could be considered refractory.

Appendix Table G8. Resolution of symptoms after initial FMT for recurrent CDI

Study	Refractory Sample	Cleared of CDI
Mellow, 2011[64]	1	1/1
Youngster, 2014 (oral)[53]	4	2/4
Zainah, 2015[46]	14	11/14

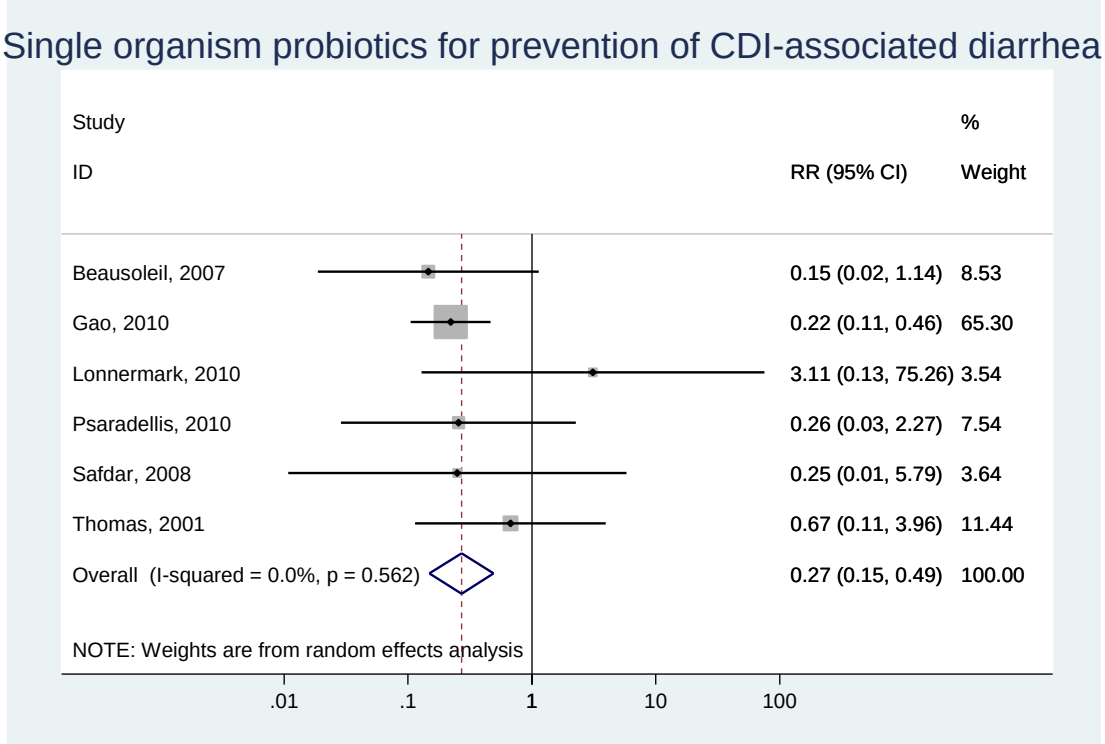
Probiotics for CDI

We identified a total of 18 studies that reported use of probiotics as adjunctive treatment for CDI: nine RCTs and one observational study were newly identified, while seven RCTs were included in the prior report. With the plethora of RCTs to provide a best evidence base, the observational study will not be discussed further.

Probiotics were administered as an adjunct to standard antibiotic treatment for CDI in all the studies. All studies included adults with mean reported age of 50 to 77 years. The studies enrolled 40 to 2981 subjects. The probiotics tested were lactobacilli species in six studies, sacchromyces species (S. boulardii) in six studies, and multiorganism in five studies: both lactobacillus and saccharomyces species in one study, lactobacillus and bifidobacterium in two studies, a four-strain preparation of *L. acidophilus*, *L. paracasei*, and bifidobacterium in one study, and VSL#3 in one study. VSL#3 contained *Bifidobacterium breve, Bidfidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii subsp. Bulgaricus,* and *Streptococcus thermophiles*. The comparator was placebo in 16 studies and standard care or no treatment in one study each. In four studies the probiotic was continued for the duration of antiobiotic therapy,[78-80,83] while in the others, the probiotic was continued for 3 to 21 days beyond antibiotic administration. Study endpoint was diagnosis of CDI and followup was limited, typically ranging from 7 days to 4 weeks, with two RCTs extending followup to 12 weeks.

For quantitative analysis, we categorized probiotics as single organism strains (lactobacillus species), *S.boulardii*, and those that contained multiple organisms. Overall, we found low-strength evidence that probiotics containing only lactobacillus organisms or multiple organisms are more effective than placebo in preventing an acute episode of CDI. We found low-strength evidence that probiotics containing *S.boulardii* given as adjunct to standard antimicrobial therapy, are comparable with placebo in preventing an episode of CDI.

Appendix Figure G21. Single organism probiotics for prevention of CDI-associated diarrhea

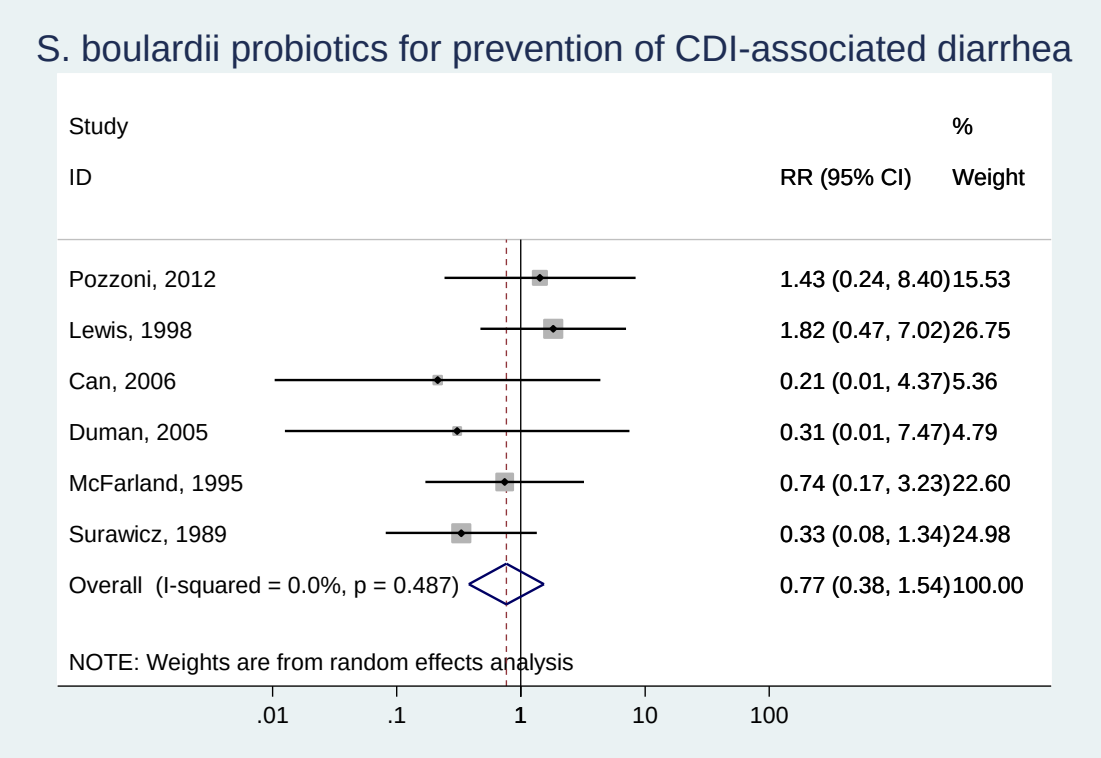


Appendix Table G9. Single organism probiotics for prevention of CDI-associated diarrhea

Study	Treatment CDI Events (percent)	Control CDI Events (percent)	Relative Risk [95% CI]
Beausoleil, 2007[78]	1/44 (2)	7/45 (16)	0.15 [0.02 to 1.14]
Gao, 2010[74]	9/171 (5)	20/84 (24)	0.22 [0.11 to 0.46]
Lonnermark, 2010[75]	1/80 (1)	0/83 (0)	3.11 [0.13 to 75.26]
Psaradellis, 2010[76]	1/216 (0.5)	4/221 (2)	0.26 [0.03 to 2.27]
Safdar, 2008[77]	0/23 (0)	1/17 (6)	0.25 [0.01 to 5.79]
Thomas, 2001[85]	2/133 (2)	3/134 (2)	0.67 [0.11 to 3.96]
Totals	14/667 (2)	35/584 (6)	0.27 [0.15 to 0.49]

CI = confidence interval

Appendix Figure G22. *S. boulardii* for prevention of CDI-associated diarrhea

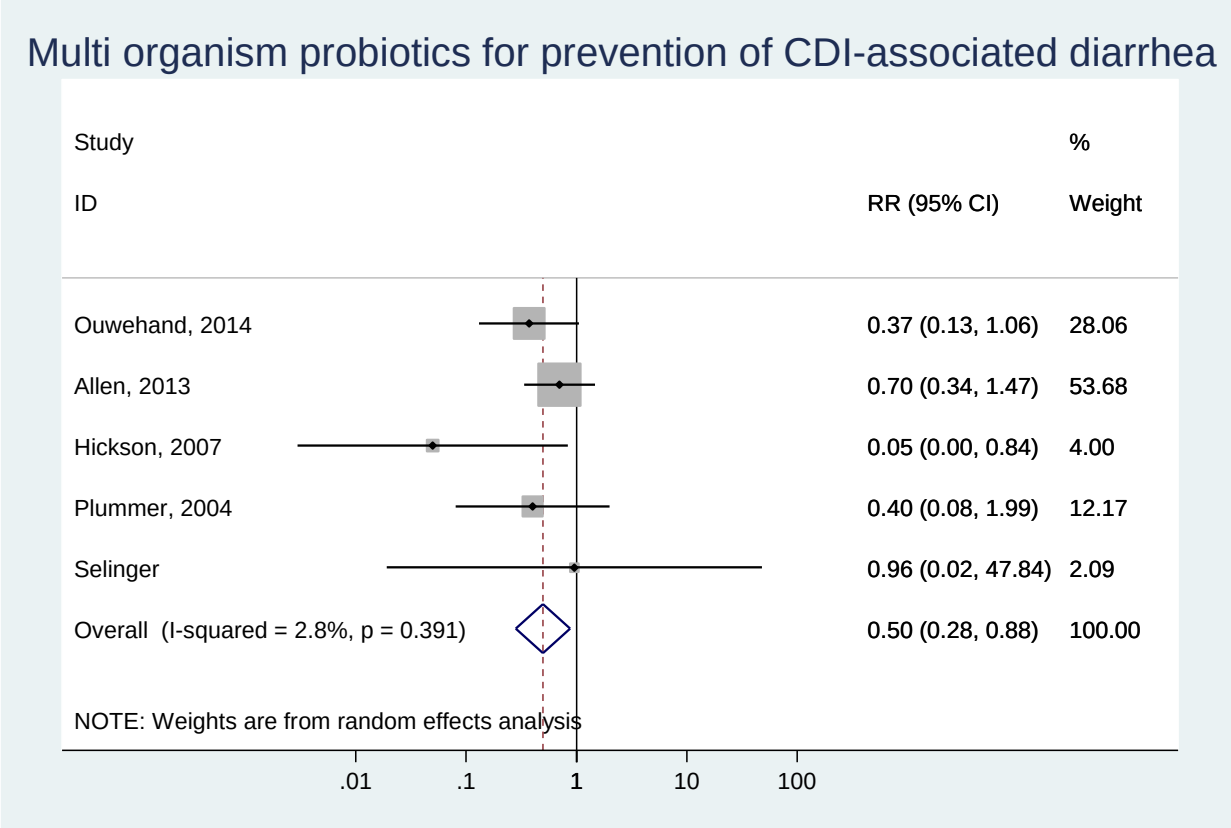


Appendix Table G10. *S. boulardii* probiotics for prevention of CDI-associated diarrhea

Study	Treatment CDI Events (percent)	Control CDI Events (percent)	Relative Risk [95% CI]
Pozzoni, 2012[73]	3/141 (2)	2/134 (1)	1.43 [0.24 to 8.40]
Lewis, 1998[86]	5/33 (15)	3/36 (8)	1.82 [0.47 to 7.02]
Can, 2006[83]	0/73 (0)	2/78 (3)	0.21 [0.01 to 4.37]
Duman, 2005[79]	0/196 (0)	1/180 (0.5)	0.31 [0.01 to 7.47]
McFarland, 1995[87]	3/97 (3)	4/96 (4)	0.74 [0.17 to 3.23]
Surawicz, 1989[88]	3/116 (3)	5/64 (8)	0.33 [0.08 to 1.34]
Totals	14/656 (2)	17/588 (3)	0.77 [0.38 to 1.54]

CI = confidence interval

Appendix Figure G23. Multi-organism probiotics for prevention of CDI-associated diarrhea



Appendix Table G11. Multi-organism probiotics for prevention of CDI-associated diarrhea

Study	Treatment CDI Events (percent)	Control CDI Events (percent)	Relative Risk [95% CI]
Ouwehand, 2014[70]	6/336 (1.8)	8/167 (4.8)	0.37 [0.13 to 1.06]
Allen, 2013[71]	12/1493 (0.8)	17/1488 (1)	0.70 [0.34 to 1.47]
Hickson, 2007[82]	0/56 (0)	9/53 (17)	0.05 [0.00 to 0.84]
Plummer, 2004[84]	2/69 (3)	5/69 (7)	0.40 [0.08 to 1.99]
Selinger, 2013[72]	0.5/117 (0.4)*	0.5/112 (0.4)*	0.96 [0.02 to 47.84]
Totals	20/2071 (1.0)	39/1889 (2)	0.50 [0.28 to 0.88]

CI = confidence interval
*Adjusted from 0 to 0.5 to facilitate analysis.

Appendix H. Strength of Evidence

The Strength of Evidence approach relies on a technique called structured implicit judgment. This technique has reviewers rate the quality of individual components and then use their judgment to produce an overall rating that takes all the components into account. For Strength of Evidence, the domains are reported out as categorical, but in fact represent concepts that should be viewed as a continuum. The final overall assessment should be consistent with domains but convey a final, global assessment. Thus, two outcomes can have two different overall assessments (that is, either insufficient or low, or low or moderate, or moderate or high) when the domains are coded identically.

Appendix Table H1. Strength of evidence assessments

Comparison	Outcomes	Finding	Study Limitations	Directness	Precision	Consistency	Evidence Rating
Diagnostics							
LAMP (1 test, 12 arms)	Sensitivity Specificity	0.95, 95% CI .090-0.97 0.98, 95% CI 0.96-0.99	Low	Direct	Precise	Consistent	High (unable to detect reporting bias)
PCR (10 tests, 31 arms)	Sensitivity Specificity	0.95, 95% CI 0.93-0.96 0.97, 95% CI 0.96-0.98	Low	Direct	Precise	Consistent	High (unable to detect reporting bias)
Toxin A/B (8 tests, 58 arms)	Sensitivity Specificity	0.70, 95% CI 0.66-0.74 0.98, 95% CI 0.97-0.98	Low	Direct	Imprecise	Consistent	Moderate (unable to detect reporting bias)
GDH (4 tests, 10 arms)	Sensitivity Specificity	0.90, 95% CI 0.78-0.96 0.94, 95% CI 0.89-0.97	Moderate	Direct	Precise	Consistent	Moderate (unable to detect reporting bias)
Test Algorithms (11 tests, 11 arms)	Sensitivity Specificity	0.73, 95% CI 0.62-0.82 1.00, 95% CI 0.99-1.00	Moderate	Direct	Imprecise	Consistent	Low
Prevention							
Antibiotic stewardship (1 systematic review, 6 studies)	CDI Incidence	Appropriate prescribing practices associated with decreased CDI					Low, per systematic review
Bathing (2 studies)	CDI Incidence		Moderate	Direct	Imprecise	Inconsistent	Insufficient
Daily cleaning with hydrogen peroxide disposable wipes (1 study)	CDI Incidence		High	Direct	Imprecise	Single Study	Insufficient
Hydrogen peroxide vapor (3 studies)	CDI Incidence		High	Direct	Imprecise	Consistent	Insufficient

Comparison	Outcomes	Finding	Study Limitations	Directness	Precision	Consistency	Evidence Rating
Pulsed ultraviolet light (1 study)	CDI Incidence		High	Direct	Imprecise	Single Study	Insufficient
Handwashing campaigns (1 moderate risk of bias study as best evidence)	CDI Incidence	Reduced CDI (rates fell from 16.75 to 9.49 cases per 10,000 bed days)	Moderate	Direct	Imprecise	Single Study	Low
Multicomponent prevention interventions (4 studies)	CDI Incidence	Sustainable over several years	Moderate	Direct	Imprecise	Consistent	Low
Treatment							
Vancomycin vs. Metronidazole 4 RCT N=872 initial N=705 recur	Initial cure	83.9% vs. 75.7%; RR 1.08, 95% CI 1.02 – 1.15	Moderate (update studies – low, with larger N; original review - high)	Direct	Precise	Consistent	High (reporting bias undetected) (Weighted by newer, lower risk of bias studies with larger N)
	Recurrent CDI	16.5% vs. 18.7%; RR 0.89, 95% CI 0.65 – 1.23	Moderate (update studies – low, with larger N; original review - high)	Direct	Imprecise	Consistent	Moderate (reporting bias undetected) (Weighted by newer, lower risk of bias studies with larger N)
Fidaxomicin vs. Vancomycin 2 RCT N=1,111 initial N=962 recur	Initial cure	RR 1.02, 95% CI 0.98-1.07	Low (low both update and original)	Direct	Imprecise	Consistent	Moderate (reporting bias undetected)
	Recurrent CDI	RR 0.55, 95% CI 0.42-0.71	Low (low both update and original)	Direct	Precise	Consistent	High (reporting bias undetected)
Effect by Disease Severity – any antibiotic 2 RCT, 1 observational	Initial cure	NS	High (update studies – moderate; original review - high	Direct	Imprecise	Consistent	Low
FMT 3 RCT, 23 case series		Resolves diarrhea and prevents relapse in patients with recurrent CDI	High (case series except 2 high risk of bias trials)	Direct	Imprecise, but numerous case series	Consistent	Low (unable to detect reporting bias) (Weighted by relatively large

Comparison	Outcomes	Finding	Study Limitations	Directness	Precision	Consistency	Evidence Rating
N=751							N, consistency of large number of case series)
		Mixed finding regarding resolving diarrhea in patients with refractory CDI (n=19)	High (all case series)	Direct	Imprecise, only 2 trials	Unknown	Insufficient
Multi-organism Probiotics vs placebo 5 RCT N=3960	Primary prevention	RR 0.48, 95%, CI 0.28-0.88	High (dominated by Allen)	Direct	Imprecise (small number of events possible for small samples)	Consistent	Low (unable to detect reporting bias) (Weighted by large N)
S. boulardii vs placebo 6 RCT N=1244	Primary prevention	RR 0.77, 05% CI 0.38-1.54	High (not dominated)	Direct	Imprecise (small number of events possible for small samples)	Consistent	Low (unable to detect reporting bias) (Weighted by relatively large N)
Single strain lactobacillus 6 RCT N=1251	Primary prevention	RR 0.27, 95% CI 0.15-0.49	High (dominated by Gao)	Direct	Imprecise (small number of events possible for small samples)	Inconsistent	Low (unable to detect reporting bias) (Weighted by relatively large N)

RR = relative risk [95 percent confidence intervals]; NS = No statistically significant difference.

Appendix I. Ongoing Studies

Appendix Table I1. Ongoing phase 3 or phase 4 studies

NCT Number	Title	Population	Interventions	Study Designs
Vaccines				
NCT01887912 Recruiting	Study of a Candidate Clostridium Difficile Toxoid Vaccine in Subjects at Risk for C. Difficile Infection	Subjects >50 age at risk for CDI and substantial unmet medical need	Vaccine	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Outcomes Assessor) Primary Purpose: Prevention
Antibiotics				
NCT02200328 Recruiting	Efficacy of Metronidazole Prophylaxis Against Clostridium Difficile-Associated Diarrhea in High Risk Adult Patients	Inpatients 55 years and older at risk for CDI	Metronidazole vs. placebo	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Caregiver) Primary Purpose: Prevention
NCT02237859 Recruiting	Vancomycin Prophylaxis in Recurrent Clostridium Difficile Infection	Adult inpatients with history of CDI within 16 weeks and treated with Flagyl or Vancomycin, or at risk	Vancomycin vs. fruit juice/placebo	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Double Blind (Subject, Caregiver, Investigator) Primary Purpose: Prevention
NCT01597505 Recruiting	Study of CB-183,315 in Patients with Clostridium Difficile Associated Diarrhea	Adults with CDI	Surotomycin vs. oral vancomycin	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor) Primary Purpose: Treatment
NCT02179658 Recruiting	A Study to Compare Safety and Efficacy of Fidaxomicin with Vancomycin in Subjects with Clostridium Difficile-associated Diarrhea (CDAD)	Japanese adult inpatients with CDI	Fidaxomycin vs. vancomycin	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor) Primary Purpose: Treatment
NCT02254967 Recruiting	Study to Compare The Efficacy of Vancomycin Therapy to Extended Duration of Fidaxomicin Therapy in the Clinical Cure of CDI in and Older Population (EXTEND)	Adults >60 with CDI	Fidaxomicin vs. vancomycin	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment
NCT01987895 Recruiting	Phase 3 Study with Cadazolid in CDAD	Adults with CDI	Cadazolid vs. vancomycin	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Investigator) Primary Purpose: Treatment

NCT Number	Title	Population	Interventions	Study Designs
FMT				
NCT02326636 Recruiting	Fecal Microbiota Transplant for Recurrent Clostridium Difficile Infection	Adult patients referred for recurrent CDI	Fecal Microbiota Transplant	Observational Model: Cohort Time Perspective: Prospective
NCT01958463 Recruiting	Transplantation of Fecal Microbiota for Clostridium Difficile Infection	Adult patients with recurrence within 6 months, or not responding to treatment	Fecal microbiota transplant	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment
NCT02301000 Recruiting	IMT for Primary Clostridium Difficile Infection	Adults with primary CDI	Intestinal microbiota therapy vs. metronidazole	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Outcomes Assessor) Primary Purpose: Treatment
Probiotics				
NCT01687543 Recruiting	Probiotics for Reduction of Infections with Clostridium Difficile in Critically Ill Patients (ProbiEnt)	Adult inpatient ICU	Dietary Supplement: L. plantarym 229 and L. plantarum 229v (+maltodextrin) vs. matodextrin	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Caregiver, Investigator) Primary Purpose: Prevention
NCT01873872 Recruiting	Evaluation of Probiotics and the Development of Clostridium Difficile Associated Diarrhea in Patients Receiving Antibiotics	Adult inpatients at risk for CDI due to antibiotic use	Theralac probiotic vs. culturelle probiotic vs. placebo	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor) Primary Purpose: Prevention
NCT02076438 Recruiting	Probiotics for Prevention of Antibiotic Associated Diarrhea and Clostridium Difficile Associated Disease	Adult inpatients with CDI	Probiotics: Culturelle (Lactobacillus Rhamnosus GG) vs. placebo	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Caregiver, Investigator) Primary Purpose: Prevention

Appendix J. References for Appendixes

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