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Point-Counterpoint: What is the optimal approach for detection of *Clostridium difficile* infection?

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Running title: Optimal approach for CDI detection

In 2010, we published an initial point-counterpoint on laboratory diagnosis of *C. difficile* infection (CDI). At that time, nucleic acid amplification tests (NAATs) were just becoming commercially available, and the idea of algorithmic approaches to CDI was being explored. Now there are numerous NAATs in the marketplace and based on recent proficiency test surveys, they have become the predominant method used for CDI diagnosis in the United States. At the same time, there is a body of literature that suggests that NAATs lack clinical specificity and thus inflate CDI rates. Hospital administrators are taking note of institutional CDI rates because they are publicly reported. They have become an important metric impacting hospital safety ratings and value-based purchasing where hospitals may have millions of dollar of reimbursement at risk. In this point-counterpoint using a Frequently Asked Question approach, Ferric Fang of the University of Washington, who has been a consistent advocate for NAAT-only approach for CDI diagnosis, will discuss the value of a NAAT-only approach, while Christopher Polage of the University of California-Davis and Mark Wilcox of Leeds University, UK, who have each recently written important articles on the value of toxin detection in the diagnosis, will discuss the impact of toxin detection in CDI diagnosis.

Frequently Asked Questions

1. Why is there so much controversy about the performance of *C. difficile* diagnostic tests?

Fang- Diagnostic tests detect either toxigenic *C. difficile* or its toxins. Many labs have switched from toxin assays to NAATs that detect toxigenic *C. difficile* in order to maximize sensitivity, as toxin assays were previously missing cases of clinically significant CDI. However some recent studies have highlighted that NAATs can be positive in colonized patients without disease, and patients with positive toxin assays may have a worse prognosis than those with a positive NAAT only (1, 2). This has renewed controversy about the optimal approach to diagnosis CDI.

Polage and Wilcox- The performance of *C. difficile* diagnostic tests is controversial for 4 reasons:

1) There is no reliable clinical or laboratory definition for CDI that accurately distinguishes true CDI from non-CDI-related symptoms in all patients (3). Most diarrhea in hospitals is not due to CDI and virtually all clinical signs and symptoms of CDI are non-specific and occur commonly in patients without CDI (4, 5). Asymptomatic *C. difficile* colonization is also common in hospitals, particularly among patients who get selected for *C. difficile* testing due to shared risk factors between colonization and CDI (6, 7). Thus, the positive predictive value of detecting toxigenic *C. difficile* in routine diarrheal samples submitted to the laboratory is low and insufficient to diagnose CDI (1-3, 7).
2) The measured performance of *C. difficile* diagnostic tests is highly dependent on the definition of CDI and ratio of CDI to colonization in the population being tested (2, 3, 8). For example, toxin tests are sensitive (and agreement with toxigenic culture is high) in patients with pseudomembranous colitis due to the high ratio of CDI to colonization in this

population (8). Conversely, toxin tests appear less sensitive in routine stool samples submitted to the laboratory due to frequent overlap of non-CDI diarrhea with *C. difficile* colonization and the lower ratio of CDI to colonization in this population (1-3, 8,9).

3) Anecdotal experiences with cases of severe CDI missed by toxin tests have promoted a desire for absolute sensitivity regardless of specificity and an erroneous belief that all patients with toxigenic *C. difficile* and diarrhea have CDI as the cause of their symptoms (9-14). Widespread misclassification of non-CDI diarrhea in patients with *C. difficile* colonization as 'CDI' has reinforced the belief that toxin tests are insensitive for CDI without systematic investigation to verify the true frequency of disease (2, 9, 11, 15-17).

4) *C. difficile* tests vary in performance accuracy, including those with the same target; for example, there are marked and sometimes significant differences in sensitivity and specificity between commercial toxin detection tests (1, 3, 9). Thus, use of less well performing tests can reinforce perceptions driven by other factors (above).

Editor's comment: The measured accuracy of any diagnostic test is dependent upon the reference test to which the diagnostic test is being compared. The American Society for Microbiology has a group that is currently working on an evidence based practice guideline for laboratory detection of *C. difficile* infection. There are over 15 different reference methods that have appeared in this literature some of which are clearly biased. This lack of a standard reference method to define *C. difficile* infection clearly complicates an already very complicated literature and there is no consensus in sight.

2. What are the effects of using nucleic acid amplification testing for *C. difficile* on *C. difficile* infection data that institutions report to public health authorities?

Fang- Since NAATs are more sensitive than toxin assays, the introduction of a NAAT will initially increase the apparent infection rate at an institution. However, this is mitigated by two factors. First, the National Health Safety Network applies a correction factor for institutions that use NAATs to diagnosis CDI, so that institutions using more sensitive diagnostic methods will not be penalized (18). Second, the greater detection of toxigenic *C. difficile* by NAATs can facilitate more effective infection control measures so that institutional infection rates subsequently decline (19-21). This has been the experience at my own institution, where several years ago our CDI rates fell within a few months of introducing NAAT and have remained low ever since. The sensitive detection of toxigenic *C. difficile* can facilitate efforts to reduce institutional transmission. That said, public health agencies must recognize that laboratory data alone cannot be used to accurately monitor CDI rates, as laboratory tests detect both colonized and infected patients.

Polage and Wilcox- When positive laboratory test results are used as the sole measure of healthcare facility-onset CDI – as is currently the case for most hospitals in the United States – NAAT-based CDI diagnosis can have a dramatic effect on the number of CDI cases institutions report publically and affect hospital reimbursement under value-based payment programs (18, 22-24). This is because NAAT-based CDI testing results in public reporting of all fecal toxin-negative samples with toxigenic *C. difficile* as positive regardless of clinical disease or treatment. Most hospitals using NAAT or GDH immunoassay plus NAAT for CDI diagnosis see an increase in the number of 'CDI cases' reported publically by 1.5 to 3-fold over rates derived from toxin tests (18, 23, 24). The NAAT-related increase is partially accounted for by an adjustment in the NHSN standardized infection ratio (SIR) calculation used to compare hospital performance, but the current adjustment does not fully correct for

the increased number of positive results at all hospitals (24). This might be appropriate if all toxin-negative patients with *C. difficile* detected by NAAT had CDI clinically, but this is not the case (2, 3, 8). Recent outcome studies show that most toxin-negative patients with *C. difficile* detected by NAAT or culture recover spontaneously without treatment and have a significantly lower rate of adverse events than toxin-positive patients; furthermore, the duration of symptoms for toxin-negative patients with *C. difficile* detected by NAAT is similar to that for *C. difficile*-negative control patients (1, 2, 25). These findings suggest that using NAAT as a standalone test for CDI diagnosis results in a considerable amount of over-diagnosis that has important clinical, financial, and reputational implications for hospitals (2, 25). For this reason, guidelines in the UK and Europe now recommend toxin testing to confirm CDI in NAAT-positive patients, and consideration of other causes for symptoms before diagnosis and treatment of CDI in toxin-negative patients (3).

3. Should GDH immunoassays be used as a screening test to determine which stool specimens should be subjected to toxin or nucleic acid amplification testing for *C. difficile*?

Fang- GDH immunoassays are more sensitive than toxin assays and can be used to screen specimens for the presence of *C. difficile* (26). However GDH is expressed by both toxigenic and non-toxigenic strains of *C. difficile*, so GDH-positive specimens must be further tested using NAAT and/or toxin assays. Such an approach is less expensive than performing NAAT on all specimens but is also less sensitive, particularly for non-027 strains (27, 28). This is not because of strain-dependent differences in GDH expression but most likely

because 027 strains tend to attain higher organism burdens. The calculated sensitivity of the GDH immunoassay is dependent on the sensitivity of the comparator method, and studies including a blinded multi-center trial using the most sensitive comparators (NAAT and toxigenic culture with detection of both spores and vegetative cells) have shown that GDH assays miss approximately 20% of specimens detected by NAAT in patients with symptomatic CDI (17, 27, 28). In short, a GDH-based algorithm is less costly but sacrifices sensitivity.

Polage and Wilcox- GDH detection is sensitive for CDI because *C. difficile* vegetative cells express and secrete GDH extracellularly, and GDH may play a role in *C. difficile* colonization *in vivo* (29). As a result, most clinical samples with toxigenic *C. difficile* detectable by culture or NAAT are positive by GDH immunoassays and virtually all samples with toxins detectable are positive for GDH (3, 9, 30). The occasional samples that are positive by NAAT but negative for GDH have a low concentration of *C. difficile* and no toxins, suggesting that these are most likely *C. difficile* carriers or patients on treatment (30). Most laboratory comparisons find that GDH immunoassays are >90% sensitive for *C. difficile,* as confirmed by two meta-analyses; a few studies report slightly lower sensitivities in the range of 83.1-87.6% (3, 9, 26). In the most recent meta-analysis, the pooled sensitivity of GDH immunoassays was 94% (95% CI, 89-97%) and 96% (95% CI, 86-99%) relative to cell cytotoxin neutralization assay and toxigenic culture, respectively; the pooled specificity was 90-96% (3). Finally, recent studies showed that GDH expression is a reliable characteristic of all common *C. difficile* strains, contradicting an earlier study, which hypothesized that differential GDH expression might explain the lower sensitivity of two-step immunoassay algorithms for some *C. difficile* ribotypes (9, 27). In summary, GDH

immunoassays are less expensive and modestly less sensitive as a screening test than some NAAT; NAAT are generally more sensitive, specific, and expensive. Samples that test positive by either method should be retested by a fecal toxin A/B immunoassay to confirm clinical CDI disease (3). Individual laboratories should choose the *C. difficile* screening test and algorithm that works best in their lab and institution.

4. What is the most cost-effective strategy for *C. difficile* diagnosis?

Fang- Although immunoassay methods are less costly for the laboratory than NAATs, a recent cost-effectiveness analysis has determined that NAAT is the most cost-effective approach from an institutional standpoint due to the $9,000 to $13,000 cost of each missed case of CDI (31). Another study found that patients diagnosed with CDI by NAAT had a two-day shorter median length of stay compared to patients diagnosed by toxin immunoassay, even though the patients did not differ with regard to co-morbidity, prior hospitalizations, laboratory parameters or mortality (32). Length of stay is an important contributor to the financial costs of CDI (33, 34), and the authors suggested that the sensitive NAAT assay might result in more timely diagnosis and treatment (32). In addition, reliance on a less sensitive diagnostic method may lead to more empiric therapy (35) and repeat laboratory testing, because clinicians lack confidence in a negative result. Thus, the use of NAAT can promote responsible antimicrobial stewardship and reduce unnecessary antibiotic and laboratory utilization.

Polage and Wilcox- The latest guidelines recommend a two or three-step algorithm as the most effective strategy to diagnose CDI and minimize over-diagnosis of *C. difficile* colonized individuals who have other causes of their diarrheal symptoms (3). The algorithm should

start with a rapid and sensitive screening test with high negative predictive value for CDI, such as a GDH immunoassay or NAAT, to minimize empiric isolation and treatment of non-CDI patients (3). Samples with a positive screening test should be retested with a toxin A/B immunoassay to identify patients with toxins, who have the highest likelihood of CDI clinically and need for treatment (3). Patients with toxigenic *C. difficile* but no fecal toxins need additional clinical evaluation to distinguish incidental *C. difficile* colonization (most patients) from CDI with a negative toxin test (fewer patients) (3). The overall sensitivity and specificity of this approach was verified in a multicenter prospective study in the UK and supported in a recent meta-analysis (1, 3). The emphasis on fecal toxin detection in this algorithm to identify patients with high (toxin-positive patients) and low (toxin-negative patients) likelihoods of clinical CDI disease is supported by outcome studies in multiple countries (1, 2, 8, 25). In terms of cost, new economic models are needed to determine which strategy is best since previous models inappropriately assumed that patients with toxigenic *C. difficile* and negative toxin tests had CDI and overlooked the costs of CDI over-diagnosis, including decreased hospital reimbursement (31, 36).

Editor's comment: A March 2016 survey of 70 members of Clinmicronet, a global list serve of doctoral clinical microbiologists showed that 55 laboratories used a NAAT only approach while 9 used a GDH/toxin screen with PCR confirmation for GDH/toxin discrepant specimens. CAP surveys of *C. difficile* testing also show a preponderance of laboratories using a NAAT only approach. Only 6 of 70 respondents used the algorithm of a GDH or NAAT screen with toxin confirmation of screen positive results described by Polage and Wilcox. Three laboratories were considering changing to this approach. One microbiologist

commented that the decision to change to this algorithm was driven by hospital administration belief that using this approach would reduce reported CDI rates.

5. Why do studies of symptoms and clinical outcomes in patients who have *C. difficile* DNA or bacteria but not toxins in stool reach such different conclusions?

Fang- NAATs and culture-based methods are more sensitive but less specific, whereas toxin assays are less sensitive but more specific. Thus, patient selection is critically important for the proper interpretation of test results. With regard to specificity, it is important to recognize that no *C. difficile* diagnostic assay is completely specific for clinical disease. Production of toxin is essential but not sufficient for disease, and even patients with high fecal toxin levels may be asymptomatic (37, 38), particularly if they have toxin-neutralizing antibodies (39). With regard to sensitivity, it is equally important to recognize that toxin assays can be negative in patients with symptomatic (and even life-threatening) CDI (10, 13, 40, 41). The insensitivity of toxin assays has been demonstrated even for cases of pseudomembranous colitis and was a major consideration leading to the development of more sensitive NAAT assays. In fact, a false-negative toxin assay is a risk factor for a fatal outcome in patients with fulminant CDI (10), and I note that one of the toxin-negative patients in the Polage study (2) "had recurrent CDI as a contributing factor to death." The bottom line is that a negative toxin assay cannot rule-out the possibility of CDI. On the other hand, the greater sensitivity of NAAT or culture-based diagnostic methods can increase the likelihood of false-positive results, particularly in patients with a low clinical probability of *C. difficile*-associated disease. Exclusion of patients who fail to meet the clinical definition

of diarrhea (or have formed stools), are receiving laxatives, or have previously tested positive, can help to reduce the number of false-positive results. The best way to avoid false-positive test results is to restrict diagnostic testing to patients who have clinical presentations consistent with CDI, and inappropriate testing can account for many of the reported instances of "overdiagnosis" (1, 2). Institutional guidelines with clear criteria for diagnostic testing can be helpful in this regard.

Some have advocated the performance of both NAAT and toxin assays to optimize patient management. However the data are conflicting. Although some studies suggest that patients with positive toxin assays have a worse prognosis than those with positive NAAT only (1, 2), many other carefully conducted studies involving more than 2,000 patients have not found toxin assays to be predictive of symptoms, disease severity, mortality, transmissibility or recurrence (15, 16, 38, 42-44). In any case, whether the detection of toxin is indicative of a worse prognosis is beside the point. The notion that a toxin assay can distinguish between colonization and infection is fundamentally flawed-- the distinction between colonization and infection is a clinical one and cannot be based on laboratory assessment alone. As Dubberke and Burnham have noted, one must "treat the patient, not the test" (45). Some patients with positive toxin assays have asymptomatic colonization (37, 38), and some patients with negative toxin assays have CDI (10, 13, 15, 16, 40-44). More than half of patients with symptomatic CDI would be missed by reliance on a toxin immunoassay (15, 16, 42-44), an unacceptably high proportion of false-negative results. Furthermore, patients with NAAT-positive/toxin-negative specimens may convert to toxin-positive on re-testing; this was observed in 21% of individuals undergoing re-testing in the Polage study (2). I recommend using a negative NAAT to rule-out the possibility of CDI and

Journal of Clinical Microbiology

a positive NAAT to indicate the possibility of CDI in a patient with a compatible clinical presentation; using this approach, toxin assays are unnecessary. Treatment decisions should be based on clinical assessment and the presence or absence of toxigenic *C. difficile*, not on the ability or failure to detect fecal toxin.

I feel compelled to point out a self-contradiction in the European guidelines that advocate toxin testing. On one hand the guidelines acknowledge that "the decision to treat CDI is ultimately a clinical decision. . . treatment should not be withheld on the basis of laboratory tests alone"-- but on the other hand, they state that "using NAAT as a stand-alone test and relying on clinical symptoms to discern patients from CDI from asymptomatic carriers is not an optimal approach. . . samples with a positive result should be tested further with a toxin EIA" (3). On what should treatment decisions be based, clinical assessment or the presence of toxin? No wonder clinicians are confused.

I strongly disagree with the suggestion that a negative toxin assay means that a patient is only colonized and not infected (1); such a simplistic approach is likely to result in the under-diagnosis of CDI and harm to patients. Although some suggest that symptomatic patients with CDI and negative toxin assays have self-limited disease that will resolve without treatment (1, 2), this cannot be concluded from the available studies, as many of the patients in these studies who had negative toxin assays received empiric treatment for CDI. Furthermore, important clinical endpoints other than mortality, such as the duration and severity of symptoms, were not measured, and the length-of-stay for culture-positive/toxin-negative patients was actually significantly longer compared to controls with both tests negative (1). Quite simply, the safety of withholding antimicrobial treatment from symptomatic patients with positive NAAT and negative toxin assay results has not been

established. Untreated patients will also continue to shed *C. difficile* with the potential to transmit infection to others, in contrast to those receiving specific antimicrobial treatment (46).

Polage and Wilcox- There is a growing consensus that most patients with *C. difficile* DNA or bacteria but no fecal toxins (i.e., toxin-negative/*C. difficile*-positive) are clinically distinct from toxin-positive patients, have better outcomes, and generally do not have CDI as a cause of their symptoms (1-3, 25). Overall, 14 of 18 studies (78%) have reported a clinical symptom or outcome difference in toxin-negative/*C. difficile*-positive patients and large studies from multiple countries have found less severe disease, a shorter duration of diarrhea, fewer CDI-related complications, and/or lower mortality in these patients (1, 2, 8, 11, 15-17, 25, 43, 44, 47-54). In several studies, outcomes were similar to negative controls despite delayed or non-reporting of NAAT or culture results and delayed or no treatment for CDI, further supporting an alternate cause of symptoms (not CDI) (1, 2, 8, 47, 53).

Nonetheless, some studies reach the opposite conclusion - that toxin-negative/*C. difficile*-positive patients have CDI and are not different from toxin-positive patients - and it is important to understand how and why this might occur (11, 15-17, 43, 49). Most of these studies were not adequately designed or powered to detect a statistical difference in rare clinical outcomes, such as CDI-related complications or mortality and erroneously interpret a non-significant *P*-value as evidence that differences do not exist (a type II statistical error) (11, 15-17, 49). Many of these studies also have significant sources of bias, which likely contributed to the authors' conclusions, including clinical reporting or reviewer knowledge of NAAT results, and automatic classification of patients with positive NAAT or culture as having CDI regardless of disease status (11, 15-17, 43, 49). Another common problem is

failure to acknowledge that many clinical signs and outcomes seen in patients tested for CDI are common and non-specific in hospitals, and so are not necessarily indicative of, or related to CDI (e.g., diarrhea, leukocytosis, ICU care) (11, 16, 49). Pre-analytic issues can also cause negative results. One study routinely placed fecal samples in Cary-Blair transport media before toxin testing, making it likely that pre-analytic dilution contributed to negative toxin EIA results and so masked the relationship between fecal toxins and CDI-related outcomes (43). In summary, there are good explanations for why some studies fail to find differences between toxin-positive and toxin-negative/*C. difficile*-positive patients, and understanding how and why such misinterpretations occur is critical to interpreting the literature in this controversial field.

Editor's comment: Because of the uncertainty of which testing approach is most accurate in predicting that a patient has CDI, it is clear that pre-analytic considerations are essential in determining who should be tested for CDI. Ensuring that tested patients have documented diarrheal disease and have not received laxatives in the past 48 hours is essential for diagnostic accuracy regardless of testing approach.

6. Will increasing the sensitivity of assays for *C. difficile* toxins in stool increase the accuracy of toxin assays?

Fang- Not necessarily. Toxin assays with increased sensitivity may reduce the incidence of false-negative results. However, *C. difficile* toxins are labile at body temperature and susceptible to inactivation by digestive enzymes (55, 56), so a completely sensitive toxin-based assay may not be feasible. Even recent "ultra-sensitive" toxin assays are still less sensitive than NAATs (57). The likelihood of clinical illness in individuals with positive

NAAT and negative ultra-sensitive toxin assay results remains to be determined. It should also be noted that improvements in the sensitivity of toxin assays will not solve the issue of false-positive results (i.e., specificity), which can be seen with any *C. difficile* diagnostic method.

Polage and Wilcox- Maybe. Higher sensitivity toxin assays will decrease the number of CDI cases 'missed' by toxin tests and bring the analytical and clinical performance closer to the traditional cell cytotoxin neutralization assay (2, 30, 57, 58). This should be a good thing. However, lowering the threshold for positive results will also decrease the specificity for CDI and lead to classifying patients with transient or low levels of toxin due to *C. difficile* colonization and antibiotic exposure as (likely erroneously) having disease (2, 57, 58). It is not known whether detecting and treating these additional patients 'labelled' as having CDI is necessary or beneficial (or possibly harmful) since most resolve their symptoms with minimal or no treatment (2). These issues could be addressed by quantifying the level of toxins to help physicians determine the likelihood that each patient has disease and warrants treatment (57, 58). In any case, the overall diagnostic accuracy will depend on the test performance characteristics *in the population being tested*. Test performance and diagnostic accuracy are affected by many factors including local testing practices, use of diarrheagenic medications, and the prevalence of CDI, *C. difficile* carriage, non-CDI diarrhea, anti-toxin antibodies, and individual *C. difficile* strains in the population (5, 7, 59). Thus, high-sensitivity toxin tests will probably improve diagnostic accuracy in hospitals/units with good *C. difficile* testing practices, a low prevalence of *C. difficile* carriage, and low prevalence of non-CDI diarrhea. However, diagnostic accuracy could easily be worse in hospitals/units with indiscriminant *C. difficile* testing and a high prevalence of *C. difficile* carriage and non-

CDI diarrhea. Overall, accurate diagnosis of CDI depends on a multitude of factors and starts at the bedside with good clinical evaluation of the likelihood of CDI and non-CDI diarrhea and appropriate sampling and testing. Having a high sensitivity toxin test will definitely be an improvement, but will not remove the need for laboratories to work with clinicians and nurses to optimize clinical evaluation, testing, and diagnosis of symptomatic patients.

7. Should the diagnostic testing strategy for *C. difficile* infection be different in oncology, transplant and other immunocompromised patients?

Fang- Immunocompromised hosts are at increased risk for CDI, and at least some studies suggest comparable clinical severity of CDI in immunocompromised patients with positive toxin assays and those with positive NAAT only (15, 49). However, as I advocate the use of NAAT to diagnosis CDI in all patients, immunocompromised patients do not require a special testing approach.

Polage and Wilcox- No. The two-step algorithm recommended in European guidelines is still preferred in oncology, transplant and immunocompromised patients (3). Moreover, diagnostic strategies based solely on detection of toxigenic *C. difficile* (e.g., NAAT only) are likely to perform worse in these patients due to high rates of treatment-related diarrhea and *C. difficile* carriage (5, 60). The lower positive predictive value of detecting toxigenic *C. difficile* when diarrheal symptoms occur in these patients reinforces the need for judicious testing, thoughtful clinical evaluation, and fecal toxin testing to maximize the accuracy of CDI diagnoses in these groups (3, 5, 60).

8. What is the significance of asymptomatic carriage of toxigenic *C. difficile*?

366 **Fang-** Asymptomatic colonized patients are an important source of *C. difficile* transmission (6,
367 61) and are at substantially increased personal risk for the eventual development of
368 symptomatic CDI (62, 63). Therefore the identification of asymptomatic carriers can
369 enhance infection control and prevention efforts. A recent study suggests that detection and
370 isolation of colonized patients can prevent hospital-acquired CDI (64), and a CDC analysis
371 has concluded that reduced transmission due to the isolation of carriers was responsible for
372 the reduction in CDI incidence (65). High-risk antibiotics (e.g., cephalosporins,
373 fluoroquinolones, clindamycin) should be avoided if at all possible in patients known to carry
374 toxigenic *C. difficile*, and the possibility of CDI should be immediately considered if
375 diarrhea, fever or other compatible symptoms develop.

376 **Polage and Wilcox-** Asymptomatic *C. difficile* carriers outnumber CDI patients by at least 5 to 1
377 in most hospitals and are likely to be an important source of nosocomial *C. difficile*
378 transmission and infection (6, 7, 62, 64). A few studies have linked asymptomatic carriers to
379 a third or more of hospital-onset CDI cases (6, 7, 61). These observations have sparked an
380 interest in screening and isolation of asymptomatic carriers as a strategy to decrease
381 healthcare-associated CDI (6, 7, 64). So far, a single before-and-after study has been
382 published with results suggesting that screening may be effective (64). However, the current
383 absence of proven interventions for asymptomatic colonization and potential ramifications of
384 isolating large numbers of patients emphasizes the need for larger, well-controlled, multi-
385 center studies to confirm the effectiveness of screening before widespread adoption (7, 64).

386 Asymptomatic *C. difficile* colonization may also be an important predisposing risk factor
387 for CDI, but the story is somewhat mixed (59, 62, 66). Studies from the 1990s associated
388 lack of symptoms after *C. difficile* acquisition with pre-existing anti-toxin antibodies and

prior asymptomatic *C. difficile* colonization with lower risk of CDI in hospitals (59, 66). These studies promoted the belief that most asymptomatic *C. difficile* carriers were immune to *C. difficile* toxins but the high rate of colonization with a non-toxigenic *C. difficile* strain (which also protects against CDI) was a potential confounder in one often mentioned review (59, 66). More recently, asymptomatic *C. difficile* colonization has been associated with an increased risk of CDI, but it is unclear if this is an artifact of NAAT testing, a change in the epidemiology and pathophysiology of CDI, or simply a reflection of differential risk according to the toxigenic status of colonizing strains (62). Hence, more work is needed to determine the relationship between asymptomatic *C. difficile* carriage and subsequent risk of CDI.

Finally, as noted above, asymptomatic *C. difficile* colonization is probably an important source of erroneous CDI diagnoses in hospitals using *C. difficile* tests with poor predictive value for CDI, as colonized patients with diarrheal symptoms due to medications, underlying disease, and other infectious agents will yield positive (misleading) results (2, 5, 7, 67-69).

Editor's comment: One of the ongoing discussions concerning *C. difficile* is if admission screening has any benefit. If asymptomatic patients are found to be colonized, they would likely to be isolated since there are data suggesting colonized patients may spread *C. difficile*. Although limiting the use of "high risk" antimicrobials in colonized patients is an attractive idea, whether it will reduce CDI infection rates is not understood. Since treatment does not reliably clear *C. difficile* in significant proportion of patients with CDI, antimicrobial clearance of carriage is also likely to be ineffective as well.

9. Much of the debate seems to be about the potential for false-positive results for *C. difficile* infection. What are the consequences of administering antibiotics to treat C. *difficile* infection to patients who are colonized, but not infected, with *C. difficile*?

Fang- Administering antibiotics to asymptomatic colonized patients will not provide a clinical benefit and will disrupt the host microbiota. The use of unnecessary antibiotics can also promote the emergence of antibiotic-resistant organisms such as VRE (vancomycin-resistant enterococci) (70).

Polage and Wilcox- Antibiotic treatment for CDI is not benign. Metronidazole and vancomycin increase the risk of colonization and infection with multi-drug resistant organisms and promote rebound overgrowth of *C. difficile* in colonized patients after antibiotic discontinuation, which can lead to prolonged shedding or active infection (CDI) (71-73). Reflexive treatment of patients with false-positive results for CDI can also lead to delayed recognition of outbreaks (e.g., norovirus) or alternative diagnoses (e.g., medication-induced diarrhea, ischemic colitis), and treatment failure (67-69). In the near future, antibiotic use in hospitals will be reported publically and hospitals will be mandated to implement antimicrobial stewardship programs to improve antibiotic use, creating additional incentives for hospitals to curb excessive/unnecessary antibiotic use. Thus, routine administration of antibiotics to patients with false-positive results for CDI has significant negative consequences for patients and hospitals.

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