*Clostridium difficile* is the most common cause of healthcare-associated infection in the US and is increasingly recognized as a pathogen in the community. *C. difficile* is considered one of the most urgent antibiotic-resistant threats to public health and can cause a variety of clinical manifestations, ranging from asymptomatic colonization, to mild diarrhea, to toxic megacolon and death. *C. difficile* infection is secondary to dysbiosis, or a condition whereby the microbiota of the gastrointestinal tract is disrupted (often secondary to antimicrobial therapy), creating an environment where *C. difficile* can flourish and cause a toxin-mediated illness. We have asked 4 experts with different roles in this field to share their thoughts on contemporary challenges in diagnosing, treating, and preventing *C. difficile* infection.

**What are some of the challenges in diagnosing *Clostridium difficile* infection (CDI)?**

**Larry Kociolek:** There are 2 general types of tests to detect *C. difficile*: (1) tests that detect free toxin, and (2) tests that detect an organism with the potential to produce toxins (i.e., toxigenic strains of *C. difficile*). Cell culture cytotoxicity neutralization assay and toxigenic stool culture, the gold standards for detection of free toxin and toxigenic strains of *C. difficile*, respectively, have very limited utility in the clinical setting because of long turnaround times (several days) and excessive labor requirements. Therefore, these assays are primarily used for research. Toxin enzyme immunoassays (EIAs) and nucleic acid amplification tests (NAATs) are the most commonly used tests for *C. difficile*. Although toxin detection is more specific for CDI (i.e., symptomatic disease), most hospitals have abandoned toxin EIAs because of potentially suboptimal sensitivity and concerns for falsely negative results. NAATs are now the most commonly used diagnostic tests for the detection of *C. difficile* in US hospitals. NAATs are easy to perform, provide rapid results, and are highly sensitive. Therefore, falsely negative results are highly unusual with NAATs. However, a limitation of NAATs is the inability to distinguish CDI and colonization.

**Erik Dubberke:** There are several challenges to diagnosing CDI. Most, if not all, of these challenges stem from the design of most studies that compare *C. difficile* diagnostic assays—the primary issue with these studies is a lack of data on the patient. Without clinical data, it is not possible to differentiate between asymptomatic *C. difficile* colonization and CDI. This lack of clinical data has had the unintended consequence of creating a strong desire to optimize sensitivity of *C. difficile* diagnostic assays. This resulted in 3 intertwined issues that afflict us today: (1) a concern that toxin assays are inadequately sensitive; (2) a mantra of “C. diff × 3” when testing for *C. difficile*; and (3) a lack of recognition of the importance of negative and positive
predictive values when interpreting C. difficile assay results. The sensitivity of culture to detect C. difficile in stool is much higher than the sensitivity of assays that detect toxin, especially if the patient is an asymptomatic carrier. This is because levels of toxin in stool are lower in asymptomatic carriers than patients with CDI. Without clinical data, it was thought those people whose stools were culture positive but toxin negative represented patients with CDI but a false-negative toxin assay. It is increasingly recognized that, when clinical data are available, the vast majority of culture positive, toxin negative stools represent asymptomatic colonization and not CDI. However, the concern over low sensitivity led to the practice of automatic repeat testing for C. difficile. This problem is rooted in the need to use positive and negative predictive values when interpreting test results. Each time a test is repeated because the last test was negative, the prevalence of CDI in the population decreases, therefore the positive predictive value also decreases. By the time one gets to the third test, there is a <20% chance a positive test represents a true positive, further demonstrating the importance of focusing on the predictive value of a test rather than the sensitivity, in most settings the negative predictive value of the first toxin EIA is >95%.

The few studies that have included clinical data based on the patient’s presentation have demonstrated that between 35% and 50% of patients tested for C. difficile do not have clinically significant diarrhea and 20% to 40% of patients recently received a laxative. We need more studies comparing C. difficile diagnostic assays that include high-quality data on both patient presentation and patient outcomes.

**How does the diagnostic assay used for C. difficile testing impact the apparent rate of CDI within a given institution?**

**Christopher Polage:** The choice of which test for laboratory confirmation of CDI has a major impact on the apparent institutional rate of CDI because up to 50% of hospitalized patients with toxigenic C. difficile and diarrhea test negative for fecal toxins by immunoassay. This paradox is often blamed on the analytical insensitivity of toxin immunoassays, but in my experience, it is more frequently due to C. difficile colonization with an alternative cause for diarrhea. That is, the detection of toxin in stool is a better predictor of symptomatic disease than a positive NAAT. Regardless, institutions using a standalone NAAT for diagnosis typically have a higher rate of “CDI” than institutions using a toxin immunoassay, and this difference can be entirely test related. Several institutions have reported a 1.5- to 2-fold jump in their measured rate of CDI after switching from a toxin immunoassay to NAAT or 3-step testing strategy of glutamate dehydrogenase (GDH) followed by NAAT if positive.

**Thomas Riley:** The C. difficile diagnostic test used can have a major impact on the apparent rate of CDI in an institution. Many studies published in the 1980s made the point that EIAs for detection of C. difficile toxins were not as sensitive as the cytotoxicity assay, and that the cytotoxicity assay was not as sensitive as toxigenic culture, yet laboratories blindly proceeded on using an insensitive EIA test. Thus, the lack of sensitivity of toxin EIAs precludes their use as standalone tests. However, unfortunately, the recent rapid uptake of highly sensitive molecular diagnostic tests has caused the pendulum to swing too far towards over-detection of C. difficile and hence over-diagnosis of disease. A switch from an EIA to a PCR-based molecular test will result in an approximate doubling of rate of detection. As is often the case, the correct answer is probably somewhere in the middle. The most important thing to remember, and many studies have made this point, is that clinical correlation is essential in the evaluation of any new test for diagnosing C. difficile-induced disease. Recent publications from the UK and US suggest that demonstrating fecal cytotoxin by C. difficile either cultured or detected by molecular methods is needed as part of any algorithm, to predict poorer outcomes for patients. What is urgently required is a diagnostic test that detects fecal cytotoxin with high sensitivity.

**How common is CDI in children?**

**Larry Kociolek:** Several epidemiologic studies using various administrative databases have documented rising incidence of CDI in children in the US over the past 15 years. These are supported by similar findings from more recent population-based studies of pediatric CDI in the US and Canada. Several pediatric studies report that the majority of children with CDI have not been hospitalized in the previous 12 weeks [i.e., community-associated (CA) CDI]. At our children’s hospital, the CDI rate has continued to rise each year since we adopted NAATs for CDI in 2009. While the rate of hospital-onset healthcare
facilities—associated (HO-HCFA) CDI has remained stable, CA-CIDI has increased significantly and concomitantly with an increased rate of CDI testing. We have also recognized frequent CDI testing among children with diarrhea who lack CDI risk factors and/or in patients with probable viral gastroenteritis (i.e., vomiting and household contacts with similar symptoms). These data suggest that understanding the true burden of CDI in children requires efforts to differentiate CDI carriers from carriers with an alternate diarrheal etiology. A large, prospective cohort study of pediatric CDI is needed to better understand the true burden of CDI in children.

Thomas Riley: Despite a lack of good evidence about C. difficile in children, several facts are irrefutable. Neonates and very young infants can have very high rates of colonization with C. difficile, and the extent to which this occurs is merely a reflection of the extent of contamination of the environment into which they are born. Breast-fed infants have less C. difficile, likely due to maternal antibody. Once infants acquire a more adult gut flora (i.e., increased numbers of Firmicutes and Bacteroidetes), C. difficile numbers decline. Rates of apparent CDI in children have increased over the last decade, both in the hospital and the community, but this is just an extension of what has been seen in adults over the same period. Clearly, more research is required before a definitive answer can be given to the question, but perhaps, in the interim, diagnostic laboratories should be a little less dismissive of CDI in very young children and infants.

How common is asymptomatic colonization with C. difficile? Should hospitals be screening patients for asymptomatic colonization?

Christopher Polage: Asymptomatic colonization with C. difficile is uncommon among healthy adults with no risk factors (<5%) but can be quite common in hospitalized patients, even at the time of admission. Rates vary between hospitals and populations but published studies suggest that 7%–21% of hospitalized adults are colonized at some point and 4%–15% are asymptomatically colonized at the time of admission. Colonization rates are higher in infants, patients with inflammatory bowel disease, and residents of long-term care, rehabilitation, and skilled nursing facilities. The classic risk factors for colonization are recent inpatient healthcare and antibiotic exposure or a history of prior CDI. More recently described risk factors include obesity, proton pump inhibitors, and virtually any condition that disrupts the colonic microbiota.

It is a more difficult question to say whether hospitals should be screening patients for asymptomatic C. difficile colonization. There is evidence that asymptomatic carriers contribute to C. difficile transmission and hospital-onset CDI in inpatient facilities. However, there is insufficient data to know how much CDI in hospitals originates from asymptomatic vs symptomatic carriers (e.g., toxin EIA-negative patients with diarrhea), prior CDI patients, environmental sources, healthcare workers, or overgrowth and toxin production from a preexisting endogenous strain. There is also a lack of studies demonstrating the effectiveness or best type of intervention(s) to decrease transmission from asymptomatic carriers. Finally, the optimal sample type and laboratory test method to screen asymptomatic individuals for C. difficile colonization need to be determined. Thus, while the role of asymptomatic carriers in C. difficile transmission is increasingly appreciated, it is too early to recommend active surveillance screening as a routine practice.

Larry Kociolek: Asymptomatic colonization is now a well-recognized phenomenon in infants and young children. It is estimated that up to 70% of infants will transiently carry C. difficile in their stool in the first year of life. By 3 years old, the colonization rate declines to 3% and remains stable throughout the remainder of childhood and into adulthood. It is likely that asymptomatic carriers can transmit C. difficile. For example, exposure to an infant has been identified as a risk factor for CA-CIDI in adults lacking significant recent exposure to outpatient healthcare settings. However, the value of screening hospitalized patients for colonization has not been determined. Routine screening of all hospitalized patients would be very costly and place an excessive burden on the microbiology laboratory. Because morbidity associated with contact precautions is well recognized, identifying carriers through routine screening and placing those patients into contact isolation would likely have deleterious downstream effects. Increased patient morbidity and reduced patient satisfaction would likely result from reduced contact between patients and healthcare workers, delays in care, noninfectious adverse events, and increased symptoms of depression and anxiety. The risk of transmission of C. difficile from asymptomatic carriers is likely to be minimal with good compliance with standard infection control measures, such as washing hands and disinfecting stethoscopes and other medical equipment between patient encounters. Therefore, it may be more cost-effective and patient centered to focus infection prevention and control efforts on improving compliance with standard precautions, which would impact transmission of all healthcare-associated pathogens.

Erik Dubberke: The colonization prevalence of patients admitted to the hospital is higher than the general population, ranging from 5% to 15%, and the colonization prevalence among patients residing in a healthcare facility is 15% to 50%. The higher prevalence and acquisition of C. difficile among people exposed to healthcare facili-
ties is likely a reflection of increased exposure to antimicrobials, which predispose to C. difficile acquisition and colonization, and increased exposure to C. difficile as a result of proximity to others who are colonized with C. difficile. The potential importance of the asymptomatic carrier as a reservoir for transmission has come to light in the recognition that only 20% to 30% of new cases of CDI can be linked to a known case of CDI when currently recommended practices to prevent transmission of C. difficile from patients with CDI are in place. These data, plus some C. difficile transmission modeling studies, suggest it may be possible to prevent new cases of CDI in healthcare facilities if transmission of C. difficile from asymptomatic carriers is prevented. However, currently there are many unknowns in regards to the risks from asymptomatic carriers. If screening for asymptomatic colonization is to be an approach to preventing CDI, the first step would be to identify a screening method that is both rapid and sensitive. At this point the quickest way to test for asymptomatic colonization is with NAATs. Unfortunately the sensitivity of these assays is only approximately 60% to 70% to detect asymptomatic colonization. The most sensitive method to test for asymptomatic colonization is with broth-enrichment selective media, but the turnaround time is at least 36 h. If either testing method was employed alone as the method of screening, there is a good possibility any intervention would have a modest impact on CDI incidence, at best.

Once the testing method is determined (as well as additional staffing needed to screen all admissions for C. difficile), the next step is to determine what to do with the information. Will all asymptomatic carriers be placed into contact precautions? If so, do hospitals have the capacity to place as many as 15% of all new admissions into contact precautions? There is also the possibility of unintended consequences. Contact precautions have been associated with adverse events and clinicians may be inclined to treat the asymptomatic colonization (which will actually increase the risk of CDI when treatment is stopped). Based on these issues and numerous unknowns, hospitals should not be screening patients for asymptomatic C. difficile colonization in the absence of a well-designed protocol adequately powered to assess its impact on CDI incidence.

What steps can or should be taken by the clinical laboratory to improve the appropriateness of C. difficile testing?

Thomas Riley: This question should be relatively easy: test the right patient and the right sample, and use the right test or combination of tests. Unfortunately, this is not as easy as it sounds, and to quote from a review about C. difficile testing published over 20 years ago, it is “still challenging.” However, there are a few simple steps that laboratories can take that will reap benefits. All diagnostic laboratories should have guidelines that ensure that only diarrheal stools are tested. There is no need for repeat testing when using a highly sensitive test, and test of cure should be discouraged. The main problem is that there is currently no single test available commercially that combines both adequate sensitivity and specificity, and therefore an algorithmic approach should be used. That approach needs to be tailored to individual laboratory requirements. There is no “one size fits all” approach here. Use an algorithm with a test with good sensitivity first and then a test that detects free toxin.

Erik Dubberke: Based on the amount of inappropriate testing done for C. difficile in the US, as well as lack of familiarity on how to interpret C. difficile assay results, there are many things that the clinical laboratory can do to improve the appropriateness of C. difficile testing. The first step is educating the clinician on when it is appropriate to test for C. difficile, e.g., limit to patients with clinically significant diarrhea, and that automatic repeat testing and test of cure are never appropriate. In addition, clinicians need to be educated regarding the fact that no C. difficile diagnostic assay is able to differentiate between asymptomatic colonization and CDI.

Education alone will likely only go so far. Often the most effective method to alter behaviors is to create barriers that limit poor practices. How the barriers are created is variable but typically involves the clinical microbiology laboratory rejecting specimens or not allowing the clinician to order automatic repeat testing or test of cure. More challenging is what the clinical microbiology laboratory can do to improve patient selection for testing. Even in settings when formed stools are rejected, a large proportion of patients with stool submitted for C. difficile testing do not have clinically significant diarrhea and/or have other modifiable causes of diarrhea. Whether or not it is possible to improve patient selection for C. difficile testing through automated methods is an area of needed investigation.

Christopher Polage: Laboratories can take many steps to increase the pretest probability of a positive test result representing CDI vs colonization and minimize clinically inappropriate testing, overtesting, overdiagnosis, and unnecessary treatment of asymptomatic or marginally symptomatic patients. The number one strategy to increase the pretest probability of disease is to limit testing to unformed or diarrheal stool specimens. All laboratories should do this by having specimen processing personnel evaluate submitted samples for consistency and reject formed samples. Other things laboratories can do to minimize false-positive results and improve test utilization
are limiting repeat testing and restricting testing of infants <1 year old. Finally, some laboratories are exploring ways to further improve the clinical predictive value of positive test results by verifying that unformed stool samples come from patients with clinically significant diarrhea vs laxative overdose, another obvious cause of diarrhea, or a single loose bowel movement without persistent symptoms.

**What is the role of antimicrobial stewardship in prevention of CDI?**

**Thomas Riley:** This is an interesting question. There have been many published examples of where institutions have modified antimicrobial prescribing practices by withdrawing an agent from the formulary or enforcing restrictions by other means, and there have been reductions in rates of CDI. Often these have occurred in outbreak situations and as part of a bundle of interventions, so it has been difficult to ascribe success to antimicrobial stewardship alone. Of course, intuitively, in an outbreak setting it makes sense to use less of an antimicrobial to which an organism is resistant, to reduce selective pressure. In the case of *C. difficile*, this may mean reducing use of cephalosporin, to which *C. difficile* is intrinsically resistant, or using fewer macrolide-lincosamide or fluoroquinolone antibiotics, which appear to be the most common drugs to which resistance develops in *C. difficile*. However, in a nonoutbreak setting does this change?

In a study modeling control bundles for *C. difficile*, we found that antimicrobial stewardship yielded meager benefits in terms of reducing the incidence of CDI, whereas reducing transmission through improvements to hygiene and sanitation had a comparatively large effect in decreasing the incidence of disease.

**Erik Dubberke:** Improving antimicrobial prescribing is a key component to any CDI prevention program. Improved antimicrobial prescribing creates herd immunity within the hospitalized population. It will decrease the number of patients who develop CDI. This, in turn, will lead to fewer patients contributing to the spread of *C. difficile*. The 2 main approaches through which antimicrobial stewardship can be leveraged in the prevention of CDI are decreasing the number of patients who are exposed to antimicrobials but do not have an indication for an antimicrobial, and, for those who do have an indication for an antimicrobial, altering antimicrobial prescribing to those associated with a lower risk of CDI when possible.

The clinical laboratory is a key component to a successful antimicrobial stewardship program.

**Larry Kociolek:** The pediatric population is unique because several studies have documented relatively low rates of antibiotic exposure preceding CDI diagnosis in children. In some studies, recent antibiotic exposure is documented to be <50% of children with CA-CDI. Because the majority of children with CDI are classified as having CA-CDI, these data suggest that, compared to adults, antibiotic stewardship programs may not be as beneficial for CDI prevention in children. However, because of the strong association between CDI and antibiotic exposure, the reliability of these data should be questioned. Many pediatric studies may be limited by inclusion of children with *C. difficile* colonization who were misdiagnosed with CDI. On the other hand, antibiotic exposures may not be fully captured in retrospective studies. The impact of ASPs on CDI rates warrants further investigation in pediatric populations.

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