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Clostridium difficile—Diagnostic and Clinical Challenges

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Guest: Dr. Christopher Polage is Associate Professor of Pathology and Infectious Diseases at the University of California Davis School of Medicine.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Clostridium difficile is the most common cause of healthcare associated infections in the United States and is increasingly recognized as a pathogen in the community. This organism is considered to be one of the most urgent antibiotic resistant threats to public health, and can cause a variety of clinical manifestations ranging from asymptomatic to mild diarrhea, to toxic megacolon, and even death.

The February 2016 issue of *Clinical Chemistry* published a Q&A session where four experts in this field shared their thoughts on contemporary challenges in diagnosing, treating, and preventing Clostridium difficile infection. We're joined by one of those experts in this podcast, Dr. Christopher Polage, Associate Professor of Pathology and Infectious Diseases at the University of California Davis School of Medicine.

Dr. Polage, you recently published a study in *JAMA Internal Medicine* about C. difficile overdiagnosis by molecular tests. That's causing quite a stir. Can you tell us a bit about this study and your findings?

Dr. Polage: Sure. The real issue is that C. difficile testing has become very controversial in recent years and this has left a lot of labs in kind of a quandary about which type of test to use for diagnosis. What we and some others observed is that there's been a lot of method comparisons in the literature but most of these didn't have clinical outcomes data and the main take home message was always that molecular tests were positive in a lot of stool samples and patients who were negative by the conventional toxin immunoassay test. But we really didn't have much data or information to go on to know what the clinical significance was of all these extra patients.

So what we wanted to know was whether these extra patients that were being detected and classified as positive

by molecular tests but who were negative by conventional toxin tests like the toxin immunoassay, whether they actually had infection or disease or really needed specific antibiotic treatment. What we did to try and address this is we basically continue to use our existing clinical toxin immunoassay test at our hospital while performing molecular tests in the background, and we did this for about two years at our academic medical center, UC Davis in Sacramento.

And we ended up at the end of it, after our sample size calculations, evaluating just over 1,400 patients and just shy of 300 patients who were positive by toxigenic culture and molecular tests, in this case PCR as well as a LAMP assay for C. difficile DNA. What turned out to be the case though, is that the clinical toxin immunoassay was positive in less than half of these patients. The basic point there is that half of the patients who were positive by the molecular tests were negative by the toxin test. So we proceeded to look at the outcomes in those patients. And what was really striking to us is that most of those patients got better without treatment and, in fact, when we compared those patients who were negative by the toxin immunoassay but positive by the molecular tests, when we compared them with people who were negative by both tests, i.e. people who basically didn't have C. difficile by any test methods, they looked very similar and we couldn't see any evidence that there were worse outcomes in the people who were only negative by the molecular test.

For us, this suggested that those people really maybe didn't need treatment for C. difficile after all, and that they might not even be infected because they seem to get better on their own without treatment. And that really caused us to question whether or not all these extra patients with positive molecular results were clinically relevant.

Bob Barrett: What are the implications of your study for labs and hospitals that perform C. difficile testing?

Dr. Polage: The big implication of this is that it causes us to take a step back and really have to decide whether or not we think all these extra patients detected by molecular tests that are negative by the traditional toxin test, whether these patients really have C. difficile infection, and whether they should be diagnosed with C. difficile infection after all, and whether they need any treatment. And our results kind of suggest that they're not. And the reason why this is such a big deal is because honestly, the stakes for many healthcare associated infections just keep getting higher in particular with C. difficile infections. There has been such a strong recognition that the rates of C. difficile infection were going up over the last decade and now C. difficile infection with

public reporting has been added to the value based purchasing model which basically is going to create a penalty system for hospital such that hospitals that have higher rates of hospital onset C. difficile infection are not going to get reimbursed at the same rate as hospitals with lower rates of Clostridium difficile infection. And this basically creates a situation where if you detect too many cases or if you're diagnosing a lot of patients who maybe don't really have infection and don't need treatment, your hospital unfortunately is going to look bad and you may end up not getting reimbursed at the same rate as other hospitals.

And the way this plays out, is that a lot of it might be then actually on the test type that you use, and so, it really has a lot of implications for laboratories and hospitals that perform C. diff testing.

Bob Barrett: Doctor, how did the results of this study compared to other studies in the literature?

Dr. Polage: Yeah. I mean, the literature has honestly been somewhat mixed in this regard. There haven't been a lot of studies that have looked at outcomes, but there have been a handful of studies prior to ours that support our results. Probably the two most significant ones is that, I guess I'll start with the previous study we did. Before we started with our prospective study, we actually did a retrospective study where we wanted to look back in all of our patients who were negative by our toxin test and make sure that we weren't missing a lot of patients with bad outcomes. We look back over about five years and we didn't see any patients who appeared to have serious complications of C. difficile. That cause us to feel comfortable that we weren't missing a bunch of patients and harming people by continuing to use the toxin test.

And then the other big study that really sort of supports our result, is there was a big four-center study published in *Lancet Infectious Diseases* in 2013 by Tim Planche and Mark Wilcox. This was basically a study out of four centers in the UK and it showed very similar results to ours, although they didn't have quite as detail the clinical data. They basically showed that all-cause mortality with lower in people who were positive by the molecular test or in their case toxigenic culture but negative by the traditional toxin assay. And what was really striking about that is that cause those investigators to actually change the recommendations for diagnosis of C. difficile infection in the United Kingdom, and basically to push the United Kingdom back towards a method where you use a sensitive screening test similar to a kind of HIV testing where you use a sensitive screening test but then you use toxin testing as a confirmatory test to

really identify which patients are most likely to have clinically significant disease and need treatment.

Now, I guess I should also acknowledge that there have been a handful of studies that disagree with ours, but none of these have really had quite the same size or have been multiple centers as our study or the UK study. And several of these, one of the real downsides of them is that they actually reported molecular test results clinically, and then they use the fact that doctors treated people with molecular test results with antibiotics as evidence that doctors thought that patients with positive molecular test results had the infection.

And so, they really created a bit of a circular reasoning there, and they had some methodological flaws that made it really difficult to interpret their results. And there's also a handful of reports out there, mostly anecdotal studies and case reports, of significant negative complications in patients with toxin immunoassays. This has been recognized for a while, but our study and the study out of UK really suggests that these events are rare, and that most patients with negative toxin test and positive molecular results probably don't have disease.

Bob Barrett: How did C. difficile testing become such a complicated and controversial issue?

Dr. Polage: Yeah. I mean this is a bit of a long story. But the real issue is that the symptoms of C. difficile infection are non-specific and even many of the signs that we associate with severe C. difficile infection. I guess the symptoms themselves, the primary symptom of C. difficile infection is diarrhea and it turns out that diarrhea in hospitalized patients--which is where most C. difficile infections occur--diarrhea is very common in hospitals and there are lots of medications that cause diarrhea, and there's lots of even underlying illnesses and even possibly tube feeds that can cause diarrhea.

And so studies that have looked at this really carefully have shown that most diarrhea in hospitalized patients is not due to C. diff. The first reason is confusing and complicated is that because it's a non-specific symptom that's caused by C. difficile and there's a lot of other causes for in the same population. The next reason is because C. difficile colonization is really common in hospitals too. It turns out that probably, in most acute care facilities across United States and elsewhere, probably somewhere in the range of 1 in 10 patients or maybe even as high as 1 in 5 patients in certain ICU and kind of high risk units may be colonized with C. difficile. This is probably about 5 to 10 times higher than the number of patients who actually have a significant infection due to C. difficile.

You kind of have a confluence of situations where you have high risk of diarrhea that's not due to C. diff caused by other things, and then you had a lot of benign C. difficile colonization that's not infection and doesn't need treatment. And where this overlap, you get a lot of patients who look like they have C. difficile because they have diarrhea and it can easily be colonized but they may not necessarily need treatment and they may not, in fact, be infected. And what we've seen over the last couple of decades is a lot of conflicting studies that have really confused people further. We know that are occasional patients who are missed by toxin immunoassay test and we know that those can occasionally be negative in patients with clinical disease. But a lot of studies make the blank assumption that anybody who is positive by culture or positive by PCR and has diarrhea automatically has C. difficile infection.

And I think that, you know, depending on if you look at clinical outcomes, this is probably just not factual and probably not true.

Bob Barrett: Okay, from a practical standpoint, what are the pros and cons of using a test for C. difficile toxins versus testing for the organism itself?

Dr. Polage: In this case here, the real question is whether you're trying to use a test that is a little bit more closely correlated with actual clinical disease, which would be the case with the toxin test. The pros of using this test are that it probably has a higher predictive value, a positive predictive value for clinical disease. And therefore, you're more likely, if the patient is symptomatic, to be detecting a patient who really needs antibiotic therapy and really needs specific treatment for C. difficile. There's probably not very much overdiagnosis with toxin tests although you still can have positive toxin tests in patients without symptoms, so it's very important to only be testing patients with real symptoms.

The downsides or the cons to toxin tests are that we do recognize that they can occasionally be negative and I don't think anybody is really denying that. I think the real question is if how often they're negative. And I think in my opinion, that has been sort of exaggerated with recent studies where they didn't look at clinical outcomes. So they can have occasional negatives, but it's probably much more rare and much less common that you have a patient who's negative and has a significant negative outcome that has really been reported or claimed in the literature.

When you kind of step over the organism-based tests, and in this group, I'm including things like the traditional

toxigenic culture where you grow C. difficile in anaerobic culture and then you prove that it's a toxigenic organism by demonstrating that that bacteria can produce toxins and culture, that's kind of the traditional reference method on the organism detection side and the more recent methods of NPCR and LAMP-based assay, different nucleic acid amplification test, either all organism-based test, and there's been one immunoassay, the detective cell wall antigen, the glutamate dehydrogenase antigen. These all detects the bacteria but not the presence of fecal toxins. And in this side here, what's good about these tests is that they're analytically better at detecting the presence of the bacteria or C. difficile, and so that makes them analytically more sensitive for C. difficile colonization. And so analytically, they're more sensitive in the laboratory when you compare them with a test for C. difficile toxins. The real issue there is that they're detecting a different target. One test is detecting toxins regardless of whether the bacteria is present and the other test is detecting bacteria regardless of whether or not the toxins are present.

These tests have the benefit of being very high sensitivity from an analytical perspective, but the real downside to these tests is that they have a lower positive predictive value and they're less specific for clinical disease. And in some cases, they're more expensive. There's really pros and cons to both sides.

Bob Barrett: Okay, doctor, I am making you the king of the world and you can now design the perfect C. difficile test. What is that test look like?

Dr. Polage: Well the first thing I would say is that this is a serious challenge. It's hard to say exactly what the perfect C. difficile test is, but I think that there's a lot of us that are starting to think that what we really need is one of two options maybe three. One of the things that a lot of people are interested in, because we still believe that toxin testing is valuable to recognize clinical disease, is that if we just had a rapid, easy to perform, but more sensitive toxin test, ideally something that was quantitative such that we could recognize whether people had below levels of toxin or high levels of toxin, that that might be one possible test that it would be more sensitive than our current toxin immunoassays and at the same time has some clinical prognostic value by quantitating toxin and that that might potentially be a very valuable C. difficile test that could both get rid of some of the downsides of current toxin tests which may not be quite as sensitive as we like but have more prognostic value at the same time than molecular tests or current tests.

The other real viable option is to take the molecular test that we've got and try and adjust the set point on those such that we make them do a better job at distinguishing clinically significant disease, which we know with patients that have higher level of Clostridium difficile DNA where you basically have got sort of a high growth or an over growth of Clostridium difficile in the colon, and we know that those organisms are more likely to be producing toxins such that we basically decrease the amount of overdiagnosis that molecular tests do, and really kind of optimize them for patients with actual clinical disease. I think making our current molecular assay quantitative and trying to derive maybe a different cut-off than they currently have such that they're not quite so analytically sensitive but that we actually tune it, if you will, for clinically significant disease, that might be another way to go.

And then I guess really quickly, I'll just say that a third thing that a lot of people are exploring, that it's unclear of how much value it's going to have, but it's really looking for evidence that the toxins and the organisms are causing some injury to the host, and that would be just correlating it with maybe evidence of inflammation from the host side or some kind of evidence that the host was actually getting injured, and then you'd have the combination of the bacteria, the toxin, and evidence of the host injury, and all of that together would tell us that this is clearly disease that needs antibiotic treatment.

It's going to be a difficult thing to solve but I think those are the directions where we need to go.

Bob Barrett: Well finally, Dr. Polage, besides the choice of test method, is there anything else laboratories can do to improve the accuracy of C. difficile test results?

Dr. Polage: Yeah, absolutely. Even before we consider switching test methods, there are several things we can do that really are likely to have a pretty significant impact on the accuracy of our results. One of the best ones and most important is to make sure that laboratories are not testing formed stool samples. It's really important because a lot of times doctors don't necessarily know what the stool sample looks like. They just get a report that the patient had a single episode of diarrhea and then they order a test for C. difficile testing. Some hospitals even have nurse-driven protocols for C. difficile testing. Laboratories sort of have to be one of the first places where we evaluate a stool samples to say, is this stool sample actually consistent with the patient having diarrheal symptoms?

It's important that we reject formed stool because that's automatic evidence that the patient may not be having

diarrhea and may not be symptomatic. And we only want to test diarrheal stools, unless the physician talks to us and says there's a real compelling reason otherwise. A second thing we can do is that we can try and work with our physician to do a better job educating them about the actual performance of our test, and emphasize to them how important it is to only test patients who have clinically significant diarrhea. What we've basically done over the last decade or so is we've been so worried about missing cases of C. difficile that we decrease our threshold for testing until in many hospitals, we were only testing patients after the first diarrheal episode and it turns out that a lot of our patients are getting laxatives and enemas and stool softeners, and things like this. And there are so many other things that can cause diarrhea that we're testing all kinds of patients that really only have one bout of diarrhea or a couple bouts of diarrhea, but they don't have any abdominal pain and they don't really have much clinical suspicion for disease.

The other thing the laboratories can do is really work closely with their physicians and try and get the word out that they really need to be careful to only test patients where there's an actual clinical significant symptom load and diarrheal load, and that they're testing patients where they actually suspect clinical C. difficile infection versus indiscriminately testing a lot of patients who don't really have significant symptoms. I think if we did those two things, we'll be a long way down the road to work better and more accurate C. difficile test results.

Bob Barrett: Dr. Christopher Polage is Associate Professor of Pathology and Infectious Diseases at the University of California Davis School of Medicine. He's been our guest in this podcast with *Clinical Chemistry* on the challenges in diagnosing, treating, and preventing Clostridium difficile infection.

I'm Bob Barrett. Thanks for listening!