TRANSCRIPT

Clostridium difficile Infection
Clinical Update: Emerging Therapies and Recurrence Prevention

October 22, 2011 1:00 PM

Boston

Dr. Dale Gerding:

Good evening, everybody. I'm Dale Gerding, I'm Professor of Medicine at Loyola University Stritch School of Medicine in Chicago and it's my pleasure to be the moderator for tonight's presentations.

I would like to welcome you to this continuing medical education symposium entitled Clostridium difficile *Infection, Clinical Update: Emerging Therapies and Recurrence Prevention*.

And I want to thank the joint sponsors of the symposium, Robert Michael Educational Institute LLC and Postgraduate Institute for Medicine. And we'd also like to thank Optimer for providing the educational grant that supports this program.

We'll have three presentations tonight. First Dr. Stephen Brecher will discuss what's new in *C. diff* diagnosis, a very, very important area right now. Then Kate Mullane, Professor at the University of Chicago, will discuss CDI pathophysiology, risk factors and recurrence. And then our last speaker will be Dr. Stuart Johnson, my colleague at Loyola, who has just recovered from his second Achilles tendon rupture, but he says he's going to stand up here and give the talk, so we'll hope that he'll be able to do that alright, and he's going to talk on CDI treatment options and infection control.

Refer to your workbook for the learning objectives and for the full disclosures that are in there by the speakers and myself.

And in order to receive continuing education credit for the program, complete the activity evaluation form that's located in the back of your workbook and hand it in at the end of the program.

At this time I'd like to introduce Dr. Stephen Brecher, who will begin the first presentation with Diagnostic Challenges and Controversies: Becoming Less Difficile. Dr. Brecher is Director of the Micro Lab at the VA Boston Healthcare System in West Roxbury, Mass.

As a reminder,The speaker presentations will feature audience response questions embedded in the talks, so when prompted to do so during the presentation please use the keypad to select your answer and again you'll have the usual eight seconds to respond.

Steve?

Dr. Stephen Brecher:

Thank you, Dale.

Good evening, welcome to my home city. I grew up about five minutes from here and I lie about 20 minutes from here now. Remember what you're eating. We've got a few things in the food, lots of good surprises.

So I want to cover in the next 25 minutes or so, diagnostic challenges and controversies.

And in fact, it is becoming less difficile to do the test, but because of some of the issues it's very difficult for people to make changes. But hopefully by the time I'm done you will consider making changes.

As a VA employee these are my opinions and not those of the VA.

So what I'm going to do is look at the guidelines, an overview of testing, rest in peace for EIA. I hope after tonight you'll go back and say why do we do this test? Going to discuss molecular tests, talk about is it valuable to do repeat testing and a phrase that I think I've coined that I'm taking credit for, mural dyslexia or inability to see the big picture because we get caught up as bean-counters and can't look beyond what we impose upon ourselves. And we really have to work in a big picture if we're going to get *C. difficile* right.

So I have a few audience response questions.

The most accurate method to diagnose *C. difficile* is EIA, GDH, a combination of EIA, GDH or PCR. You have eight seconds for this difficult question.

Okay, so good. That is the correct answer, at least as far as I'm concerned.

Specimens for *C. difficile* should be frozen before testing to release toxin, heated to select for spores, stored at room temperature or should be fresh and loose. There's another part to that but I can't say it here, I'll get in trouble. You can ask me later.

And fresh and loose, I like that. Now if you're growing it you may say heated for spores. Room temperature, the toxins are going to degrade. But if you're doing it a genetic way it may not make a difference. And frozen, I just had to make up another answer.

Okay, what do you want for CDI testing? An assay with low sensitivity and high specificity, so you get a lot of false negatives. An assay with high sensitivity and low specificity with a lot of false positives. An assay that is inexpensive, easy and quick to perform. I want to get it right, even if it's very expensive.

I'd sort of be embarrassed not to pick that I think, but you know.

So some people want to get it right and some people want it cheap, but maybe we can do both one of these days. Unfortunately that's not a choice right now.

So I'm going to start off with the guidelines. And there are two sets of guidelines I'm going to mention. The combined SHEA/IDSA guidelines, which are now a little over a year old, first part is very important, test only unformed stool. I have medical students who tell me oh, we learned in medical school that 20% of people have solid stool. The only solid stool you should consider testing is a patient who's developed ileus and someone has gone up and got it. Do not perform a test for cure. Nursing home won't take it unless we have three negatives. You're going to have positives, people can carry *C. diff*, so you don't do a test for cure. If someone's gotten better and now has diarrhea again, then you're starting over. Stool cultures are sensitive, but they're not practical. It takes two days to grow the organism and then if you grow the organism you have to do toxigenic testing. EIA is rapid, not very sensitive and is suboptimal. I'm going to even go beyond that and just say it shouldn't be done at all. The two step GDH and EIA is an interim recommendation and I will show you some data on that and it is probably, if you're moving from straight EIA on your way to PCR, it may be a way to go. More data is needed on the PCR before they can recommend – I'm well beyond that. It's now, and I'll show you enough data I hope, to convince you that we are at PCR. And repeat testing is discouraged. And I'll show you some guidelines and at some of the talks in the last few days, most people, if they go to PCR, say don't test within seven days and if it's positive there are different algorithms when and if you

should test again.

The ASM online came out with some guidelines which are a little stronger and they really said that utilizing toxin A/B EIA for *C. difficile* diagnosis is insensitive and is no longer recommended as a standalone test. The GDH antigen assays have been found to be good screening tests in many studies, but if you have a GDH, if you have positives and negatives, you have to confirm the test with something else and now you're doing a GDH, an EIA and possibly a PCR and I refer to that as difficile dancing.

A nucleic acid amplification test can be used as a standalone test and strongly agree with that and do not perform a test for cure.

So I'm Oxford, outside of Baltimore, and I saw these ducks that were all lost and it's the way I summarize *C. difficile* testing from 1984 to 2010. These were just looking for something better and we seem to be like these ducks, somewhat lost.

So how do we diagnose, what are our choices? And we have a bunch of things. We have EIA, we have what used to be one of the gold standards, cell culture neutralization assay, and then we changed to a different gold standard, which was toxigenic culture, so we'll grow the organism and then we'll see if it makes toxins by doing a cell culture neutralization assay after we grow the organisms. There were different iterations of the GDH test and it was online and then it was off and now it's popular again. And then there are some molecular tests which are either PCR – three of them are PCR and one is an isothermal amplification called LAMP.

So 45 years in microbiology and I finally got my name in print. I now have the Brecher guidelines. I mean, who gets Brecher guidelines? It's about stool. Forty-five years and this is what my legacy is going to be.

So my lab came to me and said what are our guidelines? So I came up – if it ain't loose, it's of no use. So I thought that was pretty good. And they said define loose. And so we have all these sterile sticks in the lab and so I took a stick and I put it in the stool. Sometimes it fell, sometimes it stood. So if the stick stands, the tests are banned. If the sticks fall, test them all. So that's what we use in the VA system to guide our stools.

Limit testing to one test per patient per week if you're doing PCR. If you're doing EIA, I refer to it as the Holy Trinity, the physicians order this test times 3. Why? Because it's so bad. And I'll show you that data. Do not perform a test for cure and do not perform tests on asymptomatic patients.

So looking at the issues, what are the gold standards, is it time to abandon EIA, two to three step algorithms, are we ready for PCR?

Willie Shakespeare, who I actually went to school with, wrote plate sin with gold and it goes untarnished. And it was more like the Bernie Madoff kind ___ crime. But when I thought all that and I thought of the gold standard of testing, that we really don't have a reliable gold standard. We had one, we had another. If you look at studies, a lot of the meta-analyses that are now being published are talking about using this as a gold standard versus that as a gold standard. There is no reliable gold standard. Patients can carry toxigenic strains. That's why you have to control for the specimen. So my suggested gold standard has to include a very reliable assay as well as the clinical status of the patient. And I think if you look, do they really have *C. diff*, what's the evidence, what's the test, it at least can be helpful.

Is it time to abandon EIA? Yes. The published sensitivities in the literature of 32 to 98%, and the 98% author doesn't even believe it when I talked to him a couple of months ago. So when

you have a test that has an average sensitivity of 60%, you're getting 40% false negatives. Now a lot of people said oh, I know that has patient has *C. diff*, so do it again, let's do it again, and if they run four of them and one's positive they say they have *C. diff*.

The specificity of EIA is at 92 to 100%. But I did a study and all of the low positive EIAs, not all of them, 11 out of 18 that were low positives were false positives. So I have a test with a lot of false negatives, some false positives and I'm missing 50% of my samples. This is a serious disease, this kills patients, this keeps people in hospitals for long periods of time on multiple courses of antibiotics. We've got to get it right in the laboratory. And if you can't get it right, you're part of the problem, you're not part of the solution.

What about two to three step algorithms? They've become popular, they're moving up on how many people are doing them and there were two studies that looked at using a combination of GDH, which is an EIA assay format, and an EIA for toxins A and B. And in one study, if both were positive or both were negative, the results seem to be pretty good. But 13% of the time there was discrepance, so to solve that they went to a PCR. So now you're doing three different tests in your laboratory when you could be doing one.

The other test, Susie Sharp's study looked at the same kind of stuff and had 12% that they had a resolve.

Now what some people are doing are just doing a GDH and if the GDH is negative, <u>believing it</u>. If it GDH is positive, then they're going on to a PCR. So we have a number of different algorithms going on, but I don't have anything against it, but I run a lab, I want to do one test, I want to do it quickly and I want to get it right, so I think this is on the right track and I think it's not a bad interim, but it's not where we're all going to be. If you invite me back in five years I think you'll be doing PCR by that point.

Fred Tenover did a paper that tried to explain why there was such great variation in the sensitivities of these tests. And he looked both at the EIA testing for toxins A and B and for GDH. And what they found, there are certain ribotype differences. So if you do studies on *C. diff* and collect strains from different parts of the United States, and then run EIAs or GDHs, you get funny results. It turns out there are predominant ribotypes in different areas and certain ribotypes don't give a positive EIA for toxins A and B or don't give a positive GDH. So some of the great variations that we've been seeing may have to do with ribotype differences geographically throughout at least the United States. I haven't seen studies in other parts of the world.

Right now I'm involved with a study with the University of Michigan and we're looking at six different centers through the United States. We're sending 100 to150 positive stools and they're ribotyping them and looking at some of the other things that are going on with a different *C. diff*.

So is PCR molecular ready for prime time? Well, we've got four companies that say I sure it is. There are four assays out there, BD GeneOhm, Prodesse proGASTRO, the Cepheid Xpert *C. difficile* and the Meridian Illumingene. Three of these are straight PCRs, another one is what I mentioned before as LAMP. They have different times that you can do it and I think I'm just going to show you very quickly, I don't expect that you're going to memorize this, but these are slides sent by the companies that showed the procedure.

For the BD GeneOhm, you get results in about two hours and it usually is done as a batched test, so rather than one at time you'll run them and you can get your results in two hours. The proGASTRO CD Workshop has a little bit more work and again you have to clarify and

dilute and do nucleic acid extraction. And according to their slides it's 57 minutes of hands-on time and 194 minutes total time. And again, a test like that will have to be batched. The Xpert *C. difficile* is a test so easy that even I can do it. And they've now come out with a version that not only looks for toxin B gene, but also looks for a deletion in the tcdC gene that's associated with this hypervirulent strain. And I'll have a little bit more to say about that later one. Again fairly easy to do. And Illumigene, which is the newest kid on the block, has an 11 minute sample extraction with about two minutes of hands-on time. Again you can run these in batches of ten. There's a reader and I think they will give you the reader. And this is a test that the data has looked pretty good. On all of these tests the data has looked good.

I extracted five papers from the last year and a half in the literature and difference – when you saw with EIA testing, when we saw things in the 50s and 60s, most of these are in the 90% or above sensitivity and specificity. I remind you no lab test is 100%. So if we can get in the high 90s or the mid 90s I think we're doing pretty good.

There was a meta-analysis published this month in Clinical Infectious Disease, looked at 19 studies, over 7,000 samples, over 50 years, the mean sensitivity was 90%, the mean specificity was 96%. If you take that to a more current last couple of years, those numbers are even higher. And as prevalence increases the accuracy increases.

We had a wonderful meeting that a few people put together, Ellen Jo Barren, who's sitting out there, Gary Dorne and Mike Dunne, invited 40 microbiologists to go to camp. And we had a little camp logo with a fire burning and we all went to Houston and we met for a day and a half on a number of topics and on *C. difficile* Karen Carroll chaired the session and we as a group came to a consensus that EIA for toxins A and B should not be used. Two step tests, GDH and toxins A and B with confirmations of discrepant results were acceptable, but used appropriately molecular tests are preferred.

What about doing a second test? If the first PCR is negative, should I order another PCR? Of 406 tests from 293 patients with a prior negative PCR, 396 were negative, 10 were positive, but only 3 of those were positive in less than seven days. So my basic rule is you can't have another test. I've become a czar at my VA. You want a second test? I'm the only person in the Greater Boston area that can approve it. In a year, in ten months or nine months of doing this test, I've approved eight second tests and they've all been negative. You have to beg me. I don't want to miss a case of *C. diff*, so give me some clues, tell me there's a leukocytosis, tell me the albumin is going down, the creatinine is going up, numbers of bowel movements and I'll do it. But a lot of times it's like I know that patient has *C. diff* and I look at the chart and they really don't have anything clinically to support that they have *C. diff*. So most of the time I am not going to approve a second test within seven days and I think it should be if you go to PCR testing, should be something that you incorporate into your practices.

What are the consequences of unreliable tests? First of all, you keep repeating them. Then if you have a low sensitivity, you have false negative results, so you have patients that have *C. diff*, that you don't in precautions, that you're not treating, that are spreading the spores throughout the hospital. With a low specificity with the false positive patients, you're spending money on infection control, you're treating them with antibiotics that they don't need. So really it's not just what you do in the lab that affects what happens with the patients.

What about improved tests? The first thing that will happen, and I'll warn you, don't panic, your prevalence of *C. diff* will go up. Oh, my God, look at this, we have 20 or 30% more

cases. You're now getting it right. So the true prevalence will go up, you can then spend the money on the patients that need it and avoid the other patients. You can prevent unnecessary treatment. And then you get increased productivity because you're not doing duplicate and triplicate testing. And I'm a utilization image guy, so it will improve my lab. Hey, your lab gets it right rather than your lab never gets it right, so I like that.

Which molecular test should I use? I can't tell you. Do your homework, look at costs, look at equipment, look at turnaround time, hands-on time, do you want to batch them, do you want to do it on demand, what's your prevalence, and then ask yourself what's the cost of an incorrect test.

Now another thing that came up recently with this new drug fidaxomicin, the relapse rate was different if the strain was B1, the relapse rate was similar to vancomycin in one study, a little different on a second study. But if it was not B1 or NAP1 or 027, then there was a much improved recurrence. So one of the assays can tell you if you have this hyper-virulent strain. I'm actually running the assay, but I'm not giving out the result because I'm just not sure. I still want to treat a patient based on the severity of disease rather than on the strain because I have seen patients with bad strains do very well, and patients with the not so bad strains die. So again I still think you have to look at your patient, but this is something that I'm going to keep a close eye on in the next year and may end up telling you whether it's the B1 or the NAP1 strain.

I've come up with a term, I used this in something the other morning when I asked a question at one of the *C. diff* sessions, which I think I've coined, at least I'm taking credit for, it's called mural dyslexia. And what I mean by that is we don't see the big picture because we've become bean-counters. I'm going to stay within my budget in the lab, I'm not going to spend all this money on these new tests, I'm not going to use this new drug because it's too expensive, I'm going to use these other drugs and just keep on treating them for multiple weeks.

We can save money by spending money. For any microbiologist out there, and I know there are a few, we've learned this. If I have a good idea and the test costs more money, I have to sell it, I can't just say hey, man, I've got a great idea, listen to me, I've got to go to hospital administration and explain, I've got to go to pharmacy and explain yes, if we do this right we save money. So with a more accurate method, strict specimen requirements, test volume will decrease.

I did 4,000 *C. diff* tests in 2006. I'm on line this year to do under 1,400. So my volume is going down by 60%. I've also made rules. If it ain't loose, we reject it. It takes more time to call the physician and say we're not going to do it. But if you do this, the number of tests that you do will go down. And with the correct diagnosis you get the patient treated sooner, they go home faster, and the real way to save money is length of stay. And then you spend your infection control dollars on the right patients.

So test by a molecular method. Test only unformed stool in symptomatic at-risk patients. Test only one stool per patient per week. Do not perform a test of cure. Remember no lab test is perfect, so correlate test results with patient data and clinical observation. And what I've discovered, there is light at the end of the colon.

And remember those lost ducks? Well, they've even figured it out. I turned around, I got an ice cream at the pier and went back and they're heading in the right direction and I think we're heading in the right direction.

So I want to turn it back to Dale and thank you for your time.

Dr. Dale Gerding:

Thank you very much, Steve, and thank you for being so prompt and on time.

Our next speaker, who is going to speak on *Clostridium difficile*, Pathophysiology, Risk Factors and Recurrence, is Dr. Kate Mullane. Dr. Mullane is Associate Professor of Medicine at the University of Chicago in Chicago, Illinois. That makes three out of the four people up here from Chicago, in case you haven't noticed.

And again as a reminder she will have some embedded questions for you to respond to during her presentation.

Kate?

Dr. Kathleen Mullane:

So I have some disclosures as well to offer you. I've done a lot of clinical trials for almost every company.

So we're going to talk a little bit tonight about the pathophysiology of *C. diff* and I think a lot of this is review for many people, but just to put it in a light of some of the new things that we've learned about the toxins.

So we ingest these spores daily, and if you're in the hospital and you're in an area where there may be *C. diff* spores in the area. Then unfortunately in the hospital you're exposed to many things like antibiotics and chemotherapeutic agents. And those organisms or those antibiotics and the chemotherapy will disrupt the normal gut flora and when we do that we have overgrowth of these toxic strains of *C. diff*. And when these toxic strains start overgrowing they produce their toxins. We know of three toxins, toxin A, which classically we think of as an endotoxin, toxin B, which is a cytotoxin, and then *C. diff* binary toxin, which as well is in some strains, not all, 100% of the 021 strains, about 10% overall strains. And then we know from work that Dr. Gerding has done that there are many of these strains that don't have any toxin present and may as well be protective for our gut.

So when people do have overgrowth of *C. diff* and these toxins are being produced we can have either a mild self-liming diarrhea or it can go on to become a life-threatening pseudomembranous colitis. We see toxic megacolon in some of our patients with intestinal perforations. In fact, some of our patients even have a systemic inflammatory response, which may tell us that there may be even toxin that crosses into the blood and causes a systemic reaction. But we do know that there's a systemic reaction, which we'll talk about, that occurs from the gut mucosa itself.

So what are some of the characteristics that we see in our patients who develop *C. diff*? Age over 65 is certainly a high risk factor. Patients who have multiple comorbidities, especially if they get antibiotics. Inflammatory bowel disease. If someone gets chemotherapy, especially if they're getting a bone marrow transplant, because those patients as well are getting multiple broad spectrum antibiotics. Renal impairment as we have found out, as your creatinine clearance increases, with those who have moderate to severe renal dysfunction, their risk for *C. diff* is higher and their outcomes are poorer. People with HIV as well are at increased risk for *C. diff*. Organ transplant patients, again because they may get exposure to antibiotics and have prolonged hospitalizations. And then the question of whether PPIs put patients at risk or not is always a question that comes up.

There are as well hospital predictors. So when we look at patients who are hospitalized, those patients who have had infections in the hospital are at risk for developing *C. diff*, primarily,

of course, because they're getting antibiotics. But surprisingly, some infections are more likely to cause or to put the patient at risk for *C. diff*. So Pseudomonas is much more likely than Staphylococcus, more so than Streptococcus and more so than E. coli.

We also know that those patients who have a discharge diagnosis of an infection are at higher risk for developing *C. diff*. Surprisingly, institutions where there's a low nurse to patient ratio, and a low resident to patient ratio, are as well at risk for *C. diff*. And those institutions who have long turnover, intensive care units, transplant services, long term care facilities, as well put patients at higher risk for developing *C. diff*..

We're also seeing community factors that are putting individuals at risk for *C. diff*. So if someone has had an antimicrobial agent within a month, that will portend a higher risk for *C. diff*. Use of anti-motility agents, which may mean the patient had diarrhea before they presented to you, is certainly a risk for *C. diff*. Recent hospitalizations, but not so recent, even within six months we can see an increased risk of *C. diff*. And then markers of chronic disease, meaning that the patients are going frequently to see their physicians or frequently getting hospitalized. So patients who have frequent outpatient visits, those who have GERD and it may again be related to the fact that they're getting proton pump inhibitors or that they're getting H2 blockers. And we have no real good clue on this because many patients take these as over-the-counter agents now, and so our database for understanding this is probably not totally accounted for.

Patients who have heart failure are at high risk because these patients are in the hospital and frequently seeing their physicians. And contact with infants less than 2 years old is a risk factor. And why is that? As we age we develop new receptor sites on our colon wall and the carbohydrate receptor site that *C. diff* attaches to is not present in children and so they may be colonized with this organism and shed it, but not be ill from it.

There's also the question that comes up frequently now of food-borne outbreaks or food-borne associated *C. diff*. And why is this so? Well, we know that it's isolated in the feces of animals and it's been isolated in cows, pigs, horses and chickens. Its role is unclear. We know that there is a typhylocolitis that we see in piglets and we know that there's horses that develop *C. diff* and have been reported frequently in the literature with it. But these studies are pretty small. They're in various geographic regions where they have cultured the animals from suckling through the process of them maturing and going to the finishers, which means the butcher, and so there is a lot of concern that's going with this etiology, because we're worried that these animals who are in feed lots getting antibiotics for growth, being close together, having diarrheal illnesses, are shedding these spores into our food supply, into our water supply and into the environment.

And some studies have been done looking at meat specimens in grocery stores and anywhere from 25 to 50% of the meat in the grocery store has had spores of *C. diff* in it. So I'm glad you had the sea bass today because we don't know anything about that yet, right? Okay.

So the PCR 078 is the most common strain that's been seen in the question of the food-related outbreaks. And we are seeing that much more commonly in human being as well.

So what are these toxins that are causing the issues of *C. diff*? There's multiple different clostridial toxins. The toxins for *C. diff* are in the large molecular mass group. These are direct toxins to the colon epithelium and there's multiple parallel pathways, which we'll just touch on, that go on when these toxins are activated.

So first in the gut the carbohydrate molecule allows the toxin to bind to that site. It's endocytosed into the cells and in that vacuole hydrogen molecules are accumulated. When that

happens there's a transformation in the configuration of the *C. diff* toxin and a pore is formed and the active toxin is released into the cytosol. When that happens all hell breaks loose because this toxin then can modify the small GTPases in the Rho and Ras family of enzymes and these enzymes are molecular switches. They're involved in the production and the tight junction maintenance in our cells, they're involved in gene transcription and expression, they're involved in our cell cycle and abnormalities in these or obstruction of these enzymes causes cell apoptosis.

So in parallel we're seeing enhanced permeability directly from these enzymes and these toxins of the *C. diff*. We see release of cytokines and pro-inflammatory mediators from the endocytes in the colon wall. We get neutrophil activation and a huge inflammatory reaction, which sets up the ___ reaction, and as well edema and cell wall damage. We as well see induction of apoptosis in the intestinal epithelium and the tcdA toxin primarily hits our monocytes and T cells and the tcdB toxin has direct mitochondrial apoptosis. So this all ends up resulting in necrosis and cell death of our gut wall.

Binary toxin, we don't know that much about yet. It's also called *C. difficile* transferase. It's, as I said before, it's only seen in about 10% of *C. diff* isolates, but 100% of those which are the NAP1/BI strain.

It is in the family of the actin ribosylating toxins. It's cytopathic in cell cultures. And it as well can induce tubule, microtubule formation on our cell walls, which increases the adherence of other bacteria and may be as well involved in the inflammation of the intestinal epithelial cells.

So going on from that, what are our risk factors for now patients developing severe *C. diff*? We know that the older the patient, the more likely they are to get severe *C. diff*. If someone has a white count greater than 15,000 and certainly greater than 20,000, they're at high risk for severe *C. diff*. Albumin falling, less than 2.5 grams per deciliter, a rise in creatinine, more than 1.5 times baseline or greater than 2.5 milligrams per deciliter is a high risk. The presence of a bowel obstruction or ileus. And if you do a CT on the patient and you see bowel wall thickening, stranding and ascites, that portends your patient is going to have a bad outcome.

So we developed an ATLAS score based on data from the first fidaxomicin study and then it was justified on the second study, we tested it, to see if it really worked or not. And the ATLAS score was made up of variables including age, temperature, leukocyte count, albumin and systemic concomitant antibiotics.

So when we looked at the first 516 patients from the initial fidaxomicin study we saw that if individuals had an ATLAS score of zero, that their rate of cure was 98%. However, if those patients had an ATLAS score of 7 or more, they only had a 55% chance of cure.

We then looked back on our second study and showed that this was very predictive. So when we used this ATLAS score, the full ATLAS score on our patients in the 004 study, we saw that there was a high concurrence that as the ATLAS score went up, the outcome was worse. And probably the two most common factors that were very associated with poor outcome were age and albumin in this study.

So what are some risk factors that we have for recurrence for our patients that would make us be concerned that up front this patient is going to be someone that I have to worry about and to be concerned on what therapy we're going to put them on up front. So older age, people on concomitant antibiotics, especially fluoroquinolones, although if you went to the gut microbiome study you might think it might be metronidazole as your higher risk, low serum albumin, poor performance status up front, poor immune response to toxin A and B, which we're certainly not

measuring yet, but in studies has shown us a good correlation. Concomitant treatment with antacids and proton pump inhibitors. Does that mean we should stop them right away? Probably not. Maybe if your patient is not responding or doing well, it may be something to think about. Concomitant VRE colonization, hospital-acquired disease, history of recent surgery, fecal incontinence and disruption of the microbiome and we'll talk a little bit about each of these factors.

So this is data from Pepin et al from the big outbreak that they had in Canada, looking at recurrence by age group. And as you can see, there's a significant difference in the recurrence in those patients who are over 64 years old, compared to those who are less than 64 years old.

This is wok that again came from our fidaxomicin studies, both of them put together. We looked at the outcomes of patients who had concomitant antibiotics versus those that were not on concomitant antibiotics. And this is the pooled data looking at both fidaxomicin and vancomycin and we see that the time to resolution of diarrhea was much faster in those patients who were not on concomitant antibiotics versus those who were on concomitant antibiotics. As well, those patients who had concomitant antibiotics onboard had a lower cure rate and a higher relapse rate.

So just summarizing the study, there were lower cures on those patients who had concomitant antibiotics, so 84% versus 93%. And we did see differences in the groups between those who were treated with fidaxomicin up front and those who got vancomycin, which did reach – well, did, but not very highly statistically significant, but it was.

Concomitant administration of antibiotics at any time showed a lower global cure rate, meaning up front cure and then relapse. And that difference was as well significant with fidaxomicin versus vancomycin.

So some other risks that we see in our patients are those who are in long term care facilities and we don't know why these patients are at higher risk, probably because many of them are on antibiotics or maybe because of their environment, there are a lot of spores that are around, they're exposed to many patients who are on antibiotics and as well have *C. diff*.

Up to 50% of long term care facility residents and 40% of hospitalized patients have been found to carry *C. diff* in their stool and so when you put people in the high risk area you're certainly going to see this infection occur.

This is work that was done looking at the results of patients who were able to mount an antibody response to the toxin. And when you look at the IgG and IgM, in those patients who were not able to mount an immune response, the risk of relapse was much higher than in those patients who were able to mount an immune response, and we'll certainly see what happens with the new antibody trials that will be coming soon.

We're learning more and more about the fecal microbiome. We know that this has huge issues with our immune system and with our ability to manage concurrent infections. We're born with a sterile bowel. By the time we get older there's 100 trillion different bacteria that are present with more than 200 to 1,000 different species that are present. All of these factors work with us to make our defense against infections. So we look at our gut microbiome and its effect on our Peyer's patches, on our lymph nodes in the gut, on the spleen and on immunoglobulins that we're able to produce. So the gut microbiome cross-talks with our immune system and with the intestinal epithelium in a protective manner and if we wipe that out, we put our patients at risk for developing antibiotic-associated diarrhea and certainly CDI.

The bacteria as well in our gut help us in producing short chain fatty acids, which may

have antibacterial activity, they make reactive oxygen species for us and they make bacteriocidin molecules to protect us.

When we give antibiotics we perturb that normal gut microbiome. In the short term we decrease the quantity of organisms that we have and we change the composition of those organisms. And this may last even for weeks and months after people get antibiotics. So the Bacteroides and the Firmicutes are our friends, the other organisms are not.

When we look at recurrence of *C. diff*, if someone has had a single recurrence, their likelihood of recurring is much higher. In earlier studies that were done we saw a 20% recurrence in individuals after their first episode, but I think if you look at the tolevamer studies and the studies with fidaxomicin, those rates were closer to 25% overall with vancomycin.

After a first recurrence you're much more likely to have a second recurrence and again that increases, so we have patients that have recurrence after recurrence after recurrence and we certainly need to do a lot more work looking at ways to manage these individuals.

So what are some of the strategies that we have for managing recurrent *C. diff*? Vancomycin in tapers or prolonged courses has been looked at. Saccharomyces boulardii initially looked good. In controlled studies didn't look so good. Rifaximin following vancomycin as a chaser has been worked on by Dr. Johnson. Nitazoxanide, Dr. Musher has done a lot of work on that, looking at its activity. IgG and maybe if specimens have higher concentrations of the antitoxin IgGs in them, may be why some of our individuals did better with it, but it didn't overall look any better in any of the controlled trials that have been done. Fecal transplantation has as well been used and we'll look a little bit at that and why it may work. And then fidaxomicin, we only have up-front data, we don't have any data on recurrences right now and we certainly need to look at this for a place in its therapy.

So what's a vancomycin taper? We usually start with 125 milligrams four times a day for 10 to 14 days or until the patient is no longer having diarrhea and then we start the taper. Usually it'll be 125 milligrams twice a day for seven days, then daily for seven days, then every other day for a few days, every three days for a few days. There's many different tapering regimens that have been looked, not one has been tested over another one, so you can't say which one is going to be the best. This is just one that's commonly used.

So this is a little bit about the fecal microbiome. If you look at the very middle that is a normal individual's fecal microbiome and has a lot of Bacteroides that are present there.

This is a patient who ended up having a transplant and the two bars on the left are his microbiome before he was given the transplant, so as you can see there's very few Bacteroides there and very few Firmicutes that are there. When the patient gets the donor stool up front you see that he has repopulated or as my friend says re-poopulated, his normal gut flora and has now a high concentration of the Bacteroides. In this case, longer follow-up of the patient showed that that amount of Bacteroides are protective bacteria, fell off a little bit, but is certainly in much higher concentrations than prior to his fecal transplant.

So in conclusion what can we say about some of the things that we've learned in the past few years about *C. diff*? We know that it has been an increasing for morbidity and mortality in our patients. We've learned a lot about the pathophysiology of the toxins and how they're working and we're also learning about vaccines, antibody therapy and maybe we'll have better outcomes for some of our patients by using combinations of these therapies. We know now that we can do

a better assessment at the bedside to know if patients are going to be at high risk for developing complications from *C. diff* by some simple bedside tools, looking at their albumin, looking at their fever, looking at their white count. And as well, hopefully, by learning a better understanding of the enteric microbiome we'll be able to work on things like synthetic stool to be able to re-poopulate our gut and get better responses for our patients who have *C. diff*.

So we have a few questions here. The first one is we have documented risk factors for developing CDI, include all of the following except the diagnosis of concurrent inflammatory bowel disease, a history of receiving chemotherapy, antimicrobial drug exposure in the last month or eating veal cutlets from Canada.

So you learned.

There is a high concentration of *C. diff* spores that have been seen in the calves in Canada, upwards of 80% in some of the farms that were looked at, so it is a concern, it is the 078 strain of the *C. diff*, but there have not been any associations with human outbreaks at this point in time. Elaine Petroff, one of the fellows that had worked with us in the past, has a little blip that she may be working up soon of patients that she can correlate this to, but that's not ready for prime time yet.

Question 2. The ATLAS score includes all of the following except for age, temperature, systolic blood pressure or leukocyte count.

Correct. Systolic blood pressure was not one of the things that was looked at in our ATLAS score.

Very good. Thank you.

Dr. Dale Gerding:

Thank you, Kate. And just to clarify why she was tapping me on the shoulder. I raised the age at which you're at risk for *C. diff* infection to 75 now instead of 65.

Our final speaker is my colleague at Loyola, Dr. Stuart Johnson. He's going to speak on Emerging CDI Treatment Options and Infection Control. And Stu is a Professor of Medicine at Loyola University Medical Center Stritch School of Medicine in Maywood. And again he will have some embedded questions in his talk and he's going to try to carry this off while standing. So Stu, go for it.

Dr. Stuart Johnson:

Thank you, Dale. I did tear my Achilles tendon twice. First time was tennis. But I was not taking fluoroquinolones, so just in case you wanted to know, everyone asks that.

So my disclosures. I have served as consultant for Optimer, ViroPharma, Astellas, Pfizer, Cubist and Bio-K+. I have a grant through the Veterans Department Research Service and like Steve, my opinions are not reflected from the VA.

And also this will come up later, but the FDA has approved two agents now for treating *C. diff*, that would be vancomycin and fidaxomicin. And I think I gave away, but not quite, my question.

So a patient sees you with frequent watery stools, tender abdomen, low grade fever, she just finished treatment for CDI seven days ago and she received metronidazole 500 milligrams three times a day for 14 days. Her white count is 12,000, and you recommend one, repeat metronidazole same regiment; two, repeat metronidazole but treat for three weeks; three, start

vancomycin 125 milligrams four times a day for ten days; or four, start nitazoxanide 500 milligrams twice a day for ten days.

Well, you're not following the guidelines. By the guidelines you would be well justified by treating again with metronidazole, the same regimen. The first episode of recurrence can be reliably treated the same way you treated the first episode. And I'll mention this later, but we've talked about severity, guidelines is dictating whether you start vancomycin or not, but this may be a preference as well.

So which of the following drugs does not have FDA approval for treatment of CDI? One, metronidazole; two, vancomycin capsules; three, vancomycin solution; or four, fidaxomicin.

I threw that vancomycin solution there as a trick by the way. And I was told by John Bartlett that the actual clinical trials for fidaxomicin that received FDA approval was basically the intravenous solution given orally, so just in case anyone asks.

So I would like to talk a little bit about the current SHEA/IDSA treatment guidelines, the downsides of current CDI therapies, discuss newly improved treatment option for CDI, current infection control strategies and then potential future infection control strategies.

So these were the guidelines as Dr. Brecher mentioned, that were updated last year from the SHEA/IDSA panel. In regards to treatment, the recommendation was metronidazole is the drug of choice for initial episode of mild to moderate CDI and the dose here is 500 milligrams TID for 10 to 14 days. And vancomycin is the drug of choice for initial episode of severe CDI and the dose here is 125 milligrams, not 250 or 500, four times a day for 10 to14 days. And we used a fairly simple algorithm or scoring system, if you will, for severe CDI. That would be a white count greater than 15,000 or creatinine 1.5 greater than baseline. But the key here is this is the first time we really made a recommendation based on severity, even though we don't have a perfect predictor, there are data that would suggest vancomycin is better than metronidazole for severe CDI.

So available antibiotics for treatment of CDI prior to this May included metronidazole, vancomycin, nitazoxanide, rifaximin, bacitracin, tigecycline, teicoplanin and fusidic acid. Only one of these was FDA approved and certainly tigecycline probably has the least data to support it.

Metronidazole was widely used in the United States after the 1994 CDC HICPAC caution against PO vancomycin. This was out of concern for potential resistance in Enterococci. However, we've seen decreased cure rates reported and certainly slower response times compared to vancomycin. There's a risk of neurotoxicity with prolonged use and it is inferior to vancomycin for treatment of severe CDI.

Why would that be? One of probably more important reasons for this would be the fecal drug concentrations of metronidazole. This is not an ideal drug in that it's highly absorbed. And if you look here at the fecal antibiotic concentrations, these were ten patients given vancomycin, ten given metronidazole and tested after ten days, you can see that there was only one patient that we're able actually to detect metronidazole at all. This is a log scale. Compared to most of the patients that got 125 milligram dosing of vancomycin had milligram per gram quantities in their stool.

Vancomycin, as I mentioned, was the only agent approved prior to May this year. It is highly effective and I can tell you clinically that after five, six days of treatment with vancomycin, unless they have a very severe presentation, if they still have diarrhea then something else is going on. This is a very, very effective drug. But it's higher cost than metronidazole, there's a

potential for selection of vancomycin resistance and other organisms that reside in the gut, frequent dosing, at least the way it's been studied, and then there is this 20% or more, as Kate mentioned, recurrence after treatment.

Now even though it's very effective in treating *C. diff* clinically and eradicating the organism, there is this issue of prolonging shedding. This was the same study that I mentioned before, where we randomized ten patients to vancomycin, metronidazole or placebo. And you can see here that at the end of ten days of treatment all the patients that were treated with vancomycin had cleared *C. diff* from their stool. But if you look two months later they were more likely to be positive if they had been given vancomycin than if they'd just been let alone or if they'd been treated with metronidazole. So this is one of the downsides of vancomycin, if you will.

Nitazoxanide, this is not approved, but has been studied in two prospective, randomized, comparative trials. It was compared to metronidazole and had similar response rates, but also similar recurrence rates. And as compared to vancomycin in a smaller study, only 50 patients in total, similar response rates, but really the study was too small to confirm non-inferiority or to assess recurrence rates. So this was never studied with the FDA scrutiny, if you will. So it's not approved, but it is an option for people that have failed other therapies.

Rifaximin, I may be responsible for this more than anyone, but this came out of frustration in our clinic where we had several patients that had recurrent *C. difficile*, multiple episodes despite vancomycin tapers, Saccharomyces, doing other things. So we just tried something new. We stopped vancomycin after their last treatment episode and then started rifaximin empirically, 400 milligrams twice a day for two weeks. And our first experience in the first eight patients in our clinic, seven of them had no further recurrence. Now this is not randomized, it's not blinded, but it was pretty impressive if you saw the history of these patients. One patient that did recur responded to a second course, however, the follow-up isolate was resistant in vitro to rifaximin. So this is a caution about the drug. And we reported our subsequent experience and again overall fairly positive, four of the six patients had responded.

But this resistance risk is an issue. And one study found that 36% of their isolates were resistant to rifampin. And this is a pretty good predictor of rifaximin resistance. So it is a concern. This is very much higher than most places, it's usually not that high, but it may be an indication you can't go back to the well often with this drug. So note of concern.

So what about the ideal treatment for CDI? So these are some of the characteristics that you might want in the drug. Be effective without recurrence, efficacious against virulent strains such as the BI/NAP1/027 strain, does not facilitate resistance either to *C. difficile* or other pathogens that reside in the gut, does not cross-react with clinically important antibiotics used for treating systemic infections such as vancomycin, decreases spore shedding and transmission in the hospital setting and then to top it off, has a good safety profile.

So fidaxomicin is newly available, as you know, and probably meets most of these criteria. It was approved by the FDA May 27 this year and it's indicated for the treatment of *C. difficile* associated diarrhea, AKA CDI, in adults, those over the age of 18 or older. And the recommended regimen is 200 milligrams twice a day for ten days.

Advantageous characteristics of the drug are minimal systemic absorption, it's bactericidal and it's unrelated to agents used for treatment of systemic infections. And it appears to have a more narrow spectrum of activity. And as I like to say, less collateral damage to the whole flora.

These were the data, some of the data that led to the FDA approval. This is a study that

was reported earlier this year by Tom Louie in the New England Journal. These were the results of the first randomized, multi-center Phase III trial of fidaxomicin versus vancomycin. And what is shown here is the modified to intent to treat and the per protocol results. And the primary endpoint was clinical cure at the end of ten days. And you can see the fidaxomicin in the dark blue and vancomycin in the light blue. Very similar outcomes. And this was not – fidaxomicin was statistically not inferior to vancomycin.

Now if you look at the secondary outcome, recurrence in the 30 day follow-up, you can see a difference here. There was significantly lower recurrence rate with fidaxomicin by both the modified to intent to treat analysis and the per protocol analysis. And this led to an overall global cure rate, which is simply the cure rate without recurrence. So it may be better thought of as a sustained response, if you will. And if you look at it in this way, fidaxomicin was superior to vancomycin for sustained response at the end of four weeks after treatment.

The results of the second Phase III trial has been looked at, but hasn't been reported in a peer reviewed journal, but nearly identical results from the first study were found, and so this was very convincing for the FDA and led to approval.

Now why would you see less recurrence with this drug? We think that it does have less collateral damage to the normal flora. This was one study by Tom Louie, looking at the Bacteroides group counts and feces before and after ten days of treatment, with fidaxomicin on the left and vancomycin on the right. And you can see at day zero and day 10 there was really no difference in the mean log of Bacteroides in these patients. But in vancomycin the Bacteroides counts dropped by 2 logs after ten days of treatment with vancomycin. Now this certainly is not intuitive, this drug is primarily, as you know, directed against gram-positive organisms. But at least in the stool in the concentrations that are given, it has this fairly significant effect on Bacteroides.

Now these data were just presented at ICAAC last year. These are the cure rates for fidaxomicin and vancomycin in CDI patients infected with BI and non-BI strains. These are data from both Phase III studies. And you can look here, first of all overall, that if patients were infected with BI strains, their cure rate was 86% versus 94% of patients that were cured that had non-BI strains. And you can see that this loss in efficacy was seen both with vancomycin and fidaxomicin. So there didn't appear to be superiority of fidaxomicin for treatment of the epidemic BI strain. So maybe another reason not to unblind your PCR results.

So what about the challenges with this drug? Should we treat everyone with fidaxomicin? I don't think anyone is ready to do that. I don't think that that's what should be done. But which patients should receive fidaxomicin, which would benefit the most and how will fidaxomicin fit in with the SHEA/IDSA guidelines based on treatment severity? Hospital formulary inclusion, I think most of your hospitals are struggling with this right now. And then post-approval monitoring for unanticipated side effects and evidence for resistance will be important.

There are additional potential antimicrobial agents in the pipeline. Rifampin has gone through Phase II testing and may go under Phase III trial, we don't know. This drug that just has a number has been developed by Cubist, related to daptomycin, and it looked good in Phase II trials and I understand they are going to go through Phase III studies. And there are other agents out there that may be studied as well.

So to switch gears a little bit and talk about infection control, which of the following infection control interventions for CDI is not based on clinical evidence? Glove use when caring

for patients with CDI, handwashing with soap and water after gloving when caring for patients with CDI, terminal cleaning of CDI patient room with bleach, or four, antimicrobial use restriction.

Well, most of you got it right. This is true. Handwashing with soap and water after gloving has not been tested clinically. This is a recommendation because we know in studies that alcohol does nothing to spores, if you will, on the hands. But we don't have any data that this – clinical data – that this is efficacious.

So there are infection control strategies. First, based on methods to prevent horizontal transmission, barrier precautions and then cleaning disinfection strategies, if you will. Gloves, we have data that have shown clinical efficacy. Handwashing is probable, but not studied rigorously. Gowns is completely untested. And patient cohorting we think is effective, but really is in the probable category. Cleaning, disinfection of patient room we think is probably worth it. There are data that would support this. Endoscopes as well. And rectal thermometers. Don't reuse your rectal thermometers. They have been shown to transmit *C. diff*.

So this is the only study that I'm aware of that looked at gloving for CDI intervention. And this was done at the Minneapolis VA several years ago when Dale and I were there. There were four wards that were randomized, two were the intervention wards and two were the control wards. The real intervention here was the intervention wards, gloves were readily available at the patient bedside. So you could use gloves on the other wards, but you had to go back to the nursing station to get them and we didn't facilitate it, if you will. And what was seen was significant reduction in the CDI rate on the glove wards. So you can see here in the pre-intervention time period, that the rate here for the glove wards and the control wards were such, but post-intervention the rate on the glove wards was significantly decreased, whereas the rate on the control wards was unchanged. And there was a point prevalence survey that went with this and this was also decreased on the glove wards. So I think there's pretty good evidence for using gloves.

Hand hygiene, it is an area of controversy. In routine settings alcohol-based hand gels in conjunction with isolation precautions, using gloves, may be acceptable. We did recommend as far as the SHEA/IDSA guidelines that in the setting of an outbreak or increased rate, to consider washing hands with soap and water for caring for patients with CDI. But again we don't have good data to support that and there's never been a good demonstration of introduction of alcohol hand gels and increased rates of CDI that I'm aware of. So I think if you isolate patients carefully, you use gloves, it's a toss-up whether to use soap and water for these patients. But if you're having an outbreak or increased rates, it's recommended to institute that.

This is one study, I think probably the best study still, looking at intervention with bleach. There were three different wards, again, a time series analysis. You had a pre-intervention period, a post-intervention period and rooms with CDI patients were terminally cleaned with bleach in the post-intervention period. And that was the intervention. You can see on the bone marrow transplant unit there was a significant reduction, but there was no change in the incidence in the neuro ICU or the medicine ward. And one of the implications might be that this was the ward that had the highest rate, maybe you get more benefit out of using bleach, decontamination in areas where you have more cases, if you will.

So current infection control strategies, to reduce the risk if exposed to the organism, certainly antimicrobial use restriction has been shown in several instances, particularly with

clindamycin restriction and third generation cephalosporin restriction. The data on fluoroquinolone restriction is not as solid, but we think that this is important, particularly with this new epidemic strain and needs to be tested further. Prophylactic treatment of patients receiving antimicrobials with probiotics, now I was a skeptic, and I would say I'm in the possible category here, if you will. And I'll show you some data to maybe suggest that's an issue or possibility.

What about future prevention strategies? Certainly active vaccination is being looked at. There's a toxoid vaccine that's in Phase II testing. Effective probiotics or biotherapeutics would maybe be helpful. And then adjunctive monoclonal antibodies or luminal toxin binders. Now this study with tolevamer was a big Phase III study, was a bust, and they compared this non-toxin binder straight up to antimicrobial therapy and it was not as good as even metronidazole. But it could be resurrected, if you will, and tested as adjunctive therapy.

This was a proof of concept for the toxoid vaccine. This was an earlier version of the toxoid vaccine. Three patients with multiple recurrences, were maintained on vancomycin and then given a vaccine regimen of four series. And this is looking at their serum IgG anti-toxin A and their anti-toxin B antibodies. And two of the three patients had significant rises in their antibody titer and the other one had fairly high levels to begin with. None of them had recurrences afterwards, but this is being tested now in Phase II studies. So hopefully we'll have better data in the future.

I used a little alliteration, if you will, for this. Titled this the Provocative Probiotic Primary Prevention Studies. Couldn't come up with another P. But at any rate, there was a study that was reported – Problem, there we go, I'll change the slide, Steve, thank you. So there was a study that was reported in the British Medical Journal in 2007 that was greeted with a lot of skepticism, but basically a probiotic drink that contained Lactobacillus casei, bulgaricus and Streptococcus thermophilus, was given to the antibiotic recipients, and placebo, so it was a randomized study. And the incidence of antibiotic-associated diarrhea decreased in the probiotic group and interestingly with the CDI group. Now this was challenged on a number of bases. One, they excluded maybe 95% of the patients that were screened, and including high risk antibiotics. So this was a little bit out there. But this was repeated last year in a study in China, if you will, using another probiotic combination of Lactobacillus acidophilus and Lactobacillus casei. And again they showed the same thing, a decrease in the antibiotic-associated diarrhea and CDI rates. And they actually had a kind of a dose ranging study, if you will. So one group had one of the capsules, the probiotic, the other group had two capsules, and there seemed to be a dose range study. This was done in one hospital in Shanghai in a three month period of time. We don't know much about the *C. difficile* epidemiology in China or exactly how this study was conducted. So again, these are provocative, but not proven.

Why might these work in the primary setting where they've been a miserable failure for the most part in secondary prevention? Well, this is another – going back to the fecal microbiome here – this was a nice study done by Vince Young at University of Michigan, where he took a small group of patients that had initial CDI infection, recurrent CDI infection and then three controls. And what you can see here, if you just overall look at the distribution of the major bacteria phyla, the Bacteroides, the Firmicutes, Proteobacteria and the others, you can see overall that the initial CDI group looked much more similar to the control group, but these patients with recurrent CDI had markedly disturbed distribution of these bacteria phyla. And if you look over here, this is what's called a rare faction analysis, again measuring the diversity of the fecal

microbiome, the patients with initial CDI infections in red, overlap the control group, and again it was these patients with recurrent CDI that had almost flat lines, very, very non-diverse microbiome. So it may be that the barrier for preventing *C. diff* is not as high in patients with initial infection. Those who have not had *C. diff* yet, but are on antibiotics and at risk. So more studies need to be done, but it is possible.

Now there's another approach here that was studied in our lab. This was Dr. Gerding's idea many years ago. Elaborated on work that had been done earlier, looking at prevention of CDI using a non-toxigenic strain. And this is kind of how the model works in the hamster. On day 1 you give the hamsters clindamycin and then we wait five days and give them a fully toxigenic strain. Here J-9. And the reason we wait five days is because of the susceptibility of the different strains, some are susceptible to clindamycin, some are resistant, so we wait for the washout effect. But it's a very reproducible study in our hands. And this first group of hamsters, they were colonized with M3, where they were given a high dose of M3 and you can see by day 4 they were all colonized. When they were challenged, they remained healthy, they were not infected with J-9 and were protected. Whereas the controls that just got the clindamycin and were challenged with the toxigenic strain, colonized readily and then succumbed within 48 hours.

So this has now been tested in Phase I studies and is being tested in a Phase II study and in my mind this may be the ultimate probiotic, if I could use that term. I'm not sure if the company would, but an organism that may share the same ecologic niche, metabolic niche, but a very interesting approach to this and we'll see what happens with it.

Now this study was reported last year and this was looking at monoclonal antibodies in conjunction with standard therapy. So all the patients in this study got either metronidazole or vancomycin and then were randomized to an infusion with monoclonal antibodies against toxin A and toxin B versus placebo. And what's shown here is the time to recurrence and the patients with the monoclonal antibodies, you can see that there was a marked difference. The scale here is .1, .2, .3. But significant difference in recurrence rate in those that received the monoclonal antibodies versus placebo.

So in conclusion, currently available antimicrobial agents for treatment of CDI have several limitations, particularly recurrence after initial successful treatment. Fidaxomicin, a newly approved agent, appears to be more narrow spectrum and shows promise as an improved treatment for CDI. Proven infection control interventions include gloving, antimicrobial restriction, replacing rectal thermometers with disposal units, hand hygiene and environmental contamination with sporicidal agents are likely important, but may not be needed universally. And then future prevention strategies include immunotherapy and maybe more effective probiotics.

So thank you.

Dr. Dale Gerding:

Thanks very much, Stu. And that completes our presentation.

Something that Dr. Johnson said that I just wanted to clarify. When Steve Brecher presented the guidelines it says don't treat asymptomatic patients and then Stu showed you 30 asymptomatic patients that we treated with metronidazole and vancomycin. And if you were wondering why did we do that, it's because at the time we were concerned about carriers and whether you could actually decolonize them and I think the moral of that story was don't do this

at home. It was bad because even the vancomycin-treated patients, one of them was colonized with a non-toxigenic strain. When he re-colonized he got a toxigenic strain, he ended up with a *C. diff* infection. So we did not do any favors to those asymptomatic patients and as our mentors used to tell us, it's hard to make an asymptomatic patient feel better.

Dr. Dale Gerding:

Okay, so at this time we're ready to answer your questions. So if you can please go to the microphones, I'm going to try to equitably call on people around the room. And see if we can answer your questions for you.

Audience:

Is the current ___ in 60 patients with no symptoms to explain leukocytosis, 35% had positive *C. diff* result, does that justify treatment with anti-*C. diff* just for leukocytosis without even testing or is there evidence to treat asymptomatic leukocytosis, I mean leukocytosis without *C. diff*.

Dr. Dale Gerding:

So a patient with asymptomatic leukocytosis, is it justified to treat them for *C. difficile*. Without diarrhea basically. You want to try to answer that, Stu?

Dr. Stuart Johnson:

I certainly would not treat them if they did not have symptoms consistent with CDI. I think some people have looked at this and white count may rise early and so it may be a hint that they're at risk for *C. diff* or going to develop *C. diff*, but I certainly would not use that as a criteria for treating.

Audience:

When you treat a patient with PO vanco ___ severe colitis and particularly if they have any renal insufficiency or are a dialysis patient, you can get serum levels up above 20. So I don't know the metabolism of fidaxomicin, it's not supposed to be absorbed, but let's say it is absorbed. How would it be excreted?

Dr. Dale Gerding:

Excuse me, can you repeat the last statement?

Audience:

Fidaxomicin is not supposed to be absorbed, just like PO vanco isn't, but we know PO vanco is absorbed in severe colitis. So if in severe colitis fidaxomicin is absorbed, is it hepatically metabolized or similar to vanco, will it have high serum levels in patients who are on dialysis as a risk factor for getting *C. diff* or who develop renal insufficiency as part of their severe *C. diff*.

Dr. Dale Gerding:

Stu, you want to answer that one? I don't think we know the answer yet.

Dr. Stuart Johnson:

It's being worked on. I think exactly how it's metabolized is not clear. There are some data that it is metabolized and there is a byproduct or there's a metabolite that's also seen in the stool, significant concentration. But where that is, where that happens, I don't know.

Audience:

Has anyone looked at serum levels during your severe colitis patients, to see if there's fidaxomicin there?

Dr. Dale Gerding:

We have data from the trials in patients, they're on dialysis, and their levels were measured and there is not excessive levels at this time. But you have to remember that the Phase III trials excluded the really fulminant *C. diff*, you know, possibly going to die in the next week type of patients. So I think we need to look for this and I don't think we know what to expect just yet in terms of harm to the patient that might occur if it is absorbed. But so far there hasn't been a signal that this has been a problem in the patients studied to date.

Audience:

I have two questions. One, at what level of recurrence do we tend to choose fidaxomicin? I know it's not incorporated into the guidelines yet. So for the first recurrence we might use metronidazole followed by vancomycin, the tapering dose. But at what degree of recurrence would we prefer, should we prefer to use fidaxomicin? The second question is the cost of fidaxomicin compared to vancomycin. Where are we with that?

Dr. Dale Gerding:

Stu, I think you raised all these issues in your slides, so should we treat patients with a recurrence with fidaxomicin and what about the cost?

Dr. Stuart Johnson:

It is expensive, it's a new drug and not surprisingly it's more expensive than the alternates. I don't know the answer to that question, who to use it on. I think that I would try to use it on the people that would benefit the most and I think the data that suggests that this lower recurrence rate is real. And you could use it in a very liberal definition and everyone should be using it, or you could use it in people that have multiple recurrences. And I don't think we should reserve it for people who've had multiple recurrences, but somewhere in between. And it's a good question. And different hospitals are struggling with this as far as what to ...

Audience:

Would you prefer after a patient demonstrates failure with a second dose of vancomycin? [Inaudible]

Dr. Dale Gerding:

And I think you mentioned that there is no guideline for this. The SHEA/IDSA guideline is currently under review to update it, now that fidaxomicin is available. And also now that PCR

is available. So this question is going to be struggled with by the people writing the guidelines. And I think right now none of us really have a clear answer as to how to answer your question.

Audience:

Speaking of the guidelines, I think they still recommend intravenous parenteral metronidazole in conjunction with oral vancomycin for severe *C. diff* infection, ___ seem somewhat counter-intuitive to me, acknowledging the microbiome things that Dr. Mullane referred to with ___ metronidazole. So is that under review as well?

Dr. Kathleen Mullane:

I think the concern for that is if you have a patient that has an ileus.

Audience:

It doesn't make that distinction in the guidelines. It just says severe.

Dr. Dale Gerding:

It says severe complicated. And the criteria for severe complicated is hypotension, ileus, toxic megacolon or shock. So it's for those patients only that that two drugs were recommended. And it's there because of the question of whether the oral vancomycin is going to get to the colon or not, I think. That's been a confusion, though, for a lot of people. It's also the dose that's recommended there is 500 milligrams of vancomycin four times a day. So that's an exceptional group of really quite rare patients, probably no more than 2 to 3%. So it's there for that reason and it's one of those Class C recommendations, opinion. So I don't think it's the strongest recommendation in the world either.

Audience:

Two quick questions. What's your opinion on IVIG use in fulminant disease as well as if there's any comparisons out there regards to fluoroquinolones based on the different generations, if there is any high risk or low risk with using any specific ones.

Dr. Kathleen Mullane:

So for IVIG all we have right now are studies that are not comparative, so there's no controlled trials for the use of IgG at all. Mark Miller presented last year the data that they collected from Canada with the huge outbreak of *C. diff* that they had and it was essentially we did this, we used it and our patients did okay. So at this point in time the use of IVIG can't be recommended. Where we will use it is in our patients who are hypogammaglobulinemic, in the hopes that maybe there's some IVIG or some IgG against *C. diff* in the pooled immunoglobulin that may be available. But as well, to try to just overall bolster our patients. So it's not used routinely in our patient population at all. I don't know if you use it, Stu, in your patient population.

Dr. Stuart Johnson:

Anecdotally I've seen it work and other times it doesn't. So again I don't think there's a high titer of anti-toxin antibodies. I mean, there is a fair amount of antibody in the general

population, but not high titer. So if someone were to develop a hyperimmune globulin, I think that would be worth testing. But at this time it's an expensive limited resource and I certainly – there's no data that suggests that it is effective, but ...

Dr. Dale Gerding:

The second part of that question was the different types of fluoroquinolones and risk, is that correct?

Audience:

Yeah, generally because in our institution at least, moxifloxacin still has better susceptibility to Strep pneumo as opposed to ceftriaxone. So it's a first line therapy for ___ as opposed to ceftriaxone plus azithromycin. So would you think that maybe moxi as opposed to cipro or Levaquin would maybe have less or more or equivalent or if there's any data at all, risk.

Dr. Kathleen Mullane:

It certainly has more anaerobic activity, so you're going to destroy a lot of the gut normal anaerobes that are there. There was data that was presented on the microbiome of antibiotics with the quinolones present, I don't think there's enough data to be able to say which one is better or not.

Dr. Stuart Johnson:

There have been several observations where people have made a formulary change, have gone from one fluoroquinolone to gatifloxacin or moxifloxacin and seen increased rates and variable response when they've gone back to the original fluoroquinolone. But a lot of these studies are within class. So what we think may be driving the whole thing is fluoroquinolones in general and that hasn't been studied well, but probably should be, particularly in places where you have outbreaks of this BI/NAP1 strain, which are highly fluoroquinolone resistant. And there's more data out there to suggest that they do facilitate spread of the strain.

Audience:

If fidaxomicin is narrow spectrum, why is there difference between NAP1 and non-NAP strains, the recurrence rate?

Dr. Dale Gerding:

So anybody want to take that one on?

Dr. Stephen Brecher:

I don't think we know the answer to that.

Audience:

Second thing is if you use the metronidazole or vancomycin first and then for the recurrence if you use fidaxomicin, is there any studies that still it would be preventing the recurrence, because you already disturbed your flora.

Dr. Kathleen Mullane:

Those studies are going to be coming – the initial study to get it on the market was the basic treating up front *C. diff*. So the next studies that are coming down the road are going to be multiple recurrences and some of the other populations that have been queried for how is the drug going to fit in this group. So the homework is being done on those issues.

Audience:

And the other one is the proton pump inhibitor. What is the mechanism ___?

Dr. Kathleen Mullane:

I think right now it's an association, I don't think we know the answer to that.

Dr. Stuart Johnson:

We actually are going to present some data at the clostridial pathogenesis meeting in lovely Ames, Iowa next week. But basically what we did is we tried to reproduce this in hamster using, rather than antibiotics to make the hamsters susceptible, used proton pump inhibitors. And we're trying to game the system, so we're using very high ___ of both the organism and the proton pump inhibitor. And we could see in just that setting some pass-through, increased pass-through, so we find *C. diff* in the pellets of the hamsters, but they weren't sick. But then what we did is we tried to use antibiotics and the proton pump inhibitors versus just the antibiotics in a low virulence organism and there was a hint that there was a shorter time to colonization, shorter time to disease in the patients that got antibiotics and proton pump inhibitors. But in hamsters, excuse me. And I think the story is that by themselves they're not very potent inducers of *C. diff*, but they may have some small role. And I'll ask you, has anyone ever seen a patient that started proton pump inhibitors and spontaneously developed *C. diff*? That's never been reported and I've never seen it.

Dr. Dale Gerding:

One other possible mechanism is that proton pump inhibitors have antibacterial activity, so they actually are active against Helicobacter pylori. They have MICs. So theoretically you could think they might affect the normal bacterial flora and that might be a mechanism other than they're acid neutralization. But the hamster is exquisitely sensitive and we could not see the hamsters become susceptible to *C. diff* while taking proton pump inhibitors. So we still don't have a good answer for this.

Audience:

Could you comment on the mechanism of action of fidaxomicin that seems to be effective against the *C. diff*? And also looking at the issue of the vegetative versus the spore forms. And then another corollary is why aren't you seeing some shift in the rest of the population, Bacteroides or other anaerobes? Is it very specific action?

Dr. Stuart Johnson:

The mechanism of action is pretty clear now. It certainly acts against ribosome __ RNA polymerase, if you will. Slightly different part of transcription process than the rifampin or ___. Why there's that differential, I guess the question was the differential action of fidaxomicin and vanco against Bacteroides? I think that's totally unexplained, why vancomycin has that profound

effect on Bacteroides. I just don't know the answer to that.

Audience:

One other thing is that in the metronidazole and the reason it may be recommended in severe disease, that if you do have slowdown in bowel motility or ileus, that you with a parenteral drug, you're going to have 85% that's going to have the hepatic metabolism and 10 to15% renal. So you're going to have either parent metronidazole or the metabolite that's being excreted into the bile and so that may have an effect and get down – I mean, I don't know, but there may be some beneficial effect from the IV metronidazole in the fact that it still gets into the gut.

Dr. Stuart Johnson:

If you go back and critically look at the data about metronidazole and where it goes, they talk about hepatic recirculation. I don't think it's that well studied to tell you the truth. And it's highly, highly absorbed. And there's a question about – and people have studied it in the face of *C. diff* diarrhea and they find higher concentrations in the stool when they have symptoms, when they have diarrhea. But the question is how does it get there. And is it this hepatic recirculation, is it increased bowel transit or is it secretion through inflamed mucosa. So I don't know. But it worked both for oral and IV in that one study that looked at levels.

Audience:

Certainly with hepatic failure we showed back in the70s, you have increasing levels of metronidazole serum levels with hepatic failure. So I think the hepatic component is probably important.

Audience:

I have a practical question. What about a patient who had *C. difficile*, elderly, no recurrence, readmitted to the hospital now with an infection that needs broad spectrum antibiotics, is there any data regarding using probiotics together with antibiotic treatment or even forward, giving prophylactics so quote-unquote vancomycin in this instance, to sort of prevent *C. diff*?

Dr. Kathleen Mullane:

There's absolutely no data on using another concurrent antibiotic to decrease the likelihood, no one has done those studies.

Audience:

What about probiotics?

Dr. Kathleen Mullane:

Well, Stu presented the only data that we have on probiotics and they're not a combination that we would have here, Florastor, none of those other over-the-counter probiotics have been looked at in this instance as of yet.

Dr. Stuart Johnson:

I would not use vancomycin to prevent because of the effect on the flora, I think you may actually be making them at increased risk for *C. diff*, interestingly enough. So even though it's

very effective, it has this profound effect on the flora. So I would not use that as a prophylactic agent.

Audience:
[Inaudible]

Dr. Dale Gerding:
Is there any evidence that including metronidazole in your therapy regimen will prevent patients from getting *C. diff*?

Dr. Stuart Johnson:
Yes, there is actually. There was a study of treatment for abdominal infections where it was compared, metronidazole-containing regimen versus clindamycin-containing regimen, and there seemed to be some protection in patients that got the metronidazole regimen.

Dr. Dale Gerding:
The question there was is clindamycin worse than metronidazole in terms of predisposing patients to *C. diff* and the answer was absolutely it is. So you're better off using metronidazole in your abdominal infection regimens than in using clindamycin. But clindamycin is the most notorious drug we have for causing *C. diff,* so that's a little different question than what you asked, but ...

Audience:
[Inaudible]

Dr. Dale Gerding:
You're asking a little different question for which I don't think we have an answer. But for abdominal infection we do have an answer. We've prospectively studied that.

Audience:
I am an intensivist and I would like to ask how long patient has to stay on isolation precautions because I feel that our patients are forever on isolation precautions and I feel it ___ on patient care. And the second question, should we test patients after completion of treatment, say ten days or four weeks, depends on the regimen, whether patient tests positive or not or it is just clinical resolution of the disease.

Dr. Dale Gerding:
How long to keep in isolation.

Dr. Stuart Johnson:
So the guidelines are while they have diarrhea, the kind of SHEA compendium suggested as an enhanced infection control measure that you extend that period of isolation while they're in the hospital, that's one potential infection control measure. We know that patients that have *C. diff* diarrhea have much more contamination in their environment than patients that have

resolution of the diarrhea. The other question was test of cure? No test of cure. So I'll let Steve answer that one.

Dr. Stephen Brecher:

The question is if I tell you ten days out the patient has now resolved and you get a positive test, what are you going to do with the information? You're going to retreat the patient? Or you're going to keep them on isolation? And I think we know that their shedding will be far less. So we really want to stay away from test of cure because of the potential carrier rate because we know people who get better can carry *C. diff* for an extended period of time. And finding the organism in a patient without diarrhea is just not going to be very useful and may actually get in the way. At least that's my opinion.

Audience:

First of all, the nontoxigenic strain of *C. diff* that's being used in the Phase II trials, which company is doing that?

Dr. Dale Gerding:

The company is ViroPharma.

Audience:

And nobody mentioned stool transplants at all.

Dr. Stuart Johnson:

Oh, yeah, Kate did.

Dr. Kathleen Mullane:

What would you like to know about them?

Audience:

What do you think?

Dr. Kathleen Mullane:

I think that they're very effective. The big issue that we have, and actually there was a meeting in Canada where they essentially shut down all stool transplants except for one study and one site because of manufacturing process, so I think that's the biggest issue. You can't standardize it. And so that's the problem that we have. The other issue that we have is who's going to do it. You need to, in doing a stool transplant, either decide you're going to do it, letting the patient do it at home, or you're going to try and get it through your IRB with a protocol or through your hospital administration with a protocol, and that all patients have to have HIV testing, syphilis testing, hepatitis testing, etc. up front, from the donor. And then you also add the $5,000 cost of a colonoscope. So there is an article that is in Clinical Gastroenterology and Hepatology that looks at giving individuals the recipe to do it at home. You need to make sure that it's a fairly large volume, but not so much that the patient is going to have the urge to defecate as soon as the volume is placed. And that they hold it as long as possible. There still is

about a 10% failure risk with patients doing it by enema. So colonoscopy is probably anecdotally better. But those studies haven't been done in a controlled fashion at this point in time.

Dr. Stephen Brecher:

But the other good thing is I think, I don't know, we all came up with this line separately or together or – if you get it from, say, somebody healthy, usually a spouse, it's the only time you should take crap from a spouse.

Dr. Katherine Mullane:

And we have had issues that the spouse is colonized, so we have looked at patients who – we had one patient where we tried to get it done, the husband was positive and the son was positive for *C. diff* and they did have low grade diarrhea as well and then it took a huge, you know – for getting both of them treated beforehand. And by the time we got both of them treated we had treated the individual, the wife, and she was better, so she didn't need the stool transplant after all that.

Dr. Dale Gerding:

Cost of goods is low, but the quality control leaves something to be desired.

Audience:

So do you think we'll be using the PCR test in the future to help control hospital outbreaks in the sense of screening asymptomatic people to isolate the asymptomatic carriers to control the ...

Dr. Stephen Brecher:

You're making my blood pressure go up. That's a question. I'll take it one step further. With the CMS Medicare look at well – one of the reasons for doing MRSA screens is well, that patient had MRSA, so we can still pay for them. They didn't get it in the hospital. And when we get to the next level, will we start screening everyone for *C. diff*, and I don't know the answer, but it may be as a way of – if Medicare is no longer going to pay for nosocomial *C. diff* and most of it is nosocomial *C. diff*, then, well, let's screen the patients to make sure they didn't come in with it. And then do we get even more sophisticated and say well, can we tell you anything more than B1 or non-B1 and how far do you go with it and when does the legal – so I'm not anxious to start doing it. It's also right now PCR is relatively expensive and I can justify the cost based on getting the diagnosis sooner and treating sooner. When we start doing it on everyone, I don't know. But it's a great question.

Dr. Dale Gerding:

Thank you for your attention this evening and we hope you found the program informative and useful in your practice.

END