Critical stressors in the microbiology lab: four Cs

Anne Paxton

April 2015—If you ask average patients what infectious diseases they worry about contracting during a hospital stay, Ebola may top the list, perhaps followed by MRSA and HIV. But ask clinical microbiologists what has been keeping them up at night lately, and those pathogens aren't the ones they cite.

Microbiology laboratory directors think about keeping the most deadly and the most expensive diseases treated in the hospital under control. So you're likely to hear mention of at least three Cs: *Clostridium difficile, Candida,* carbapenem-resistant Enterobacteriaceae, and perhaps a fourth, Creutzfeldt-Jakob disease.

That "C" could also stand for "costliest"—in terms of morbidity and mortality, money, and sheer travail.

Dr. Schuetz

From the perspective of the microbiology laboratory, three kinds of microbes present unique challenges, says Audrey Schuetz, MD, MPH: pathogens with a lot of potential to be transmitted to patients nosocomially, pathogens that affect patient movement in the hospital, and pathogens with a high risk of transmissibility to laboratory workers.

Dr. Schuetz, who is associate director of clinical microbiology laboratory services at New York Presbyterian Hospital and associate professor at Weill Cornell Medical College, names C. *diff* as one of her biggest concerns. According to a study reported in the *American Journal of Infection Control*, patients with C. *diff* infection are twice as likely to be readmitted to the hospital as patients without the deadly diarrheal infection, and end up with a week's longer length of stay than other readmissions (Olsen MA, et al. 2015;43[4]:318–322).

For the microbiology laboratory, one of the main issues relates to the logistics of testing these patients. "So many people come in saying they have diarrhea and a C. *diff* is ordered, but then the patient has to sit and wait for a bowel movement so a specimen can be tested, which can take up to a day or longer. Not many hospitals have the space to accommodate patients for the wait," says Dr. Schuetz, a member of the CAP Microbiology Resource Committee.

Her laboratory uses highly sensitive PCR to test for C. *diff*, but the tests have to be batched. "We went from doing this once a day, which was unacceptable for our emergency department, to twice during the day shift, and now once each shift. But the turnaround time is still not what the ED is expecting or wants." While there has recently been talk of a point-of-care test becoming available, she says there is concern about whether the ED would use the test appropriately. Tests on formed stools or the wrong patient population can produce a lot of false-positives, she notes.

"When we went back to our records for at least a year, up to 15 percent of stools sent to us were deemed inappropriate for testing, whether because they are pediatric stools where there is a low likelihood of actual disease, or because they are totally formed stools. A lot of the people running point of care aren't necessarily microbiologists, so I think it's very important that microbiology is involved in these discussions about bringing in infectious-disease–related tests."

Unexpected positives, she notes, have also been a problem with a multiplex viral respiratory panel the hospital recently brought in. “We were really excited about bringing in a multiplex assay to pick up all these viruses we weren’t even screening for before, like parainfluenzas, but some of those pathogens do require contact or different types of infection control precautions, and they put a lot of patients in a situation in which they had to wait for an appropriate room to open up.”

Perversely, adding a more accurate diagnostic test can get hospital departments in trouble. “When we went to C. *diff* PCR, our rates went up because we had been using a less sensitive assay and missing cases before. So infection control had to do some explaining. They were on the hot seat a little bit, but then the rates plateaued.”

Dr. Beavis

One of the least understood aspects of C. *diff* is the number of asymptomatic carriers, says Kathleen G. Beavis, MD, director of the microbiology and immunology laboratories at University of Chicago Medical Center and interim director of clinical laboratories at University of Chicago Medicine. Many more people are colonized with C. *diff* than have the disease, Dr. Beavis notes. But whether they are infectious is unclear. She was intrigued to discover recently that two studies 20 years apart, one done in the last two years, which screened patients on admission to the hospital or to a particular ward, found that 20 percent of patients were colonized with C. *diff* on admission.

“We always assumed that people with diarrhea are more infectious because they have diarrhea and are spewing out the organisms. But with asymptomatic carriers, we’re not aware, and we don’t take special infection control precautions like extra cleaning when that patient leaves the hospital room,” Dr. Beavis says.

If the patient does shed into the environment, the organism will survive; it’s very hardy. “So we have to be creative and explore the role of the asymp-tomatic carrier if we want to reduce our rates.”

It’s important when hospitals are using PCR to test patients for C. *diff* to make sure they have diarrhea, because unlike culture, PCR will only find the C. *diff* organism—not whether the organism the patient has is toxigenic. “With PCR, we won’t know if the gene is turned on and making the toxin or if the person is asymptomatic,” Dr. Beavis says.

Most hospitals have internally tracked their C. *diff* rates for a long time, but more hospitals are paying a lot of attention to C. *diff* now that hospital-acquired infection rates are posted publicly, says Dr. Beavis, who is a member of the CAP Microbiology Resource Committee. This was a move she expected would start for C. *diff* once MRSA rates were subject to public reporting several years ago.

Under federal reporting law starting this year, if a patient is diagnosed in the laboratory with C. *diff* on the fourth day or later of a stay, it is presumed to be a hospital-acquired infection and it counts against the hospital. Medicare reimbursement can also be cut. “Like all hospitals, we’re focused on patient care,” Dr. Beavis says. “But we also want our numbers to look good. So we’ve been looking at our rates of C. *diff* and seeing what we can do to get the rates down.”

One measure they’ve taken along those lines is to try to get clinicians not to order a test for C. *diff* once the patient has been put on a laxative. “The other thing is if someone comes into the hospital with diarrhea, we’re encouraging the physicians to place the order and the nurses to collect the specimen so C. *diff* can be diagnosed in the first three days. Then it doesn’t count as a hospital-acquired infection.”

Several different approaches to C. *diff* can be taken, says Lance R. Peterson, MD, director of microbiology and

infectious diseases research and associate epidemiologist for NorthShore University HealthSystem in Chicago's northern suburbs. At NorthShore, "Our approach has been to have the highest sensitivity test to detect as many people with toxin-producing organisms as we can, and then drive those rates as low as possible by infection control and environmental hygiene. So we just use the PCR test in each hospital 24 hours a day." The patients aren't isolated while the test is being performed, but the result comes back in less than two hours.

There's a lot of benefit to having a rapid test for C. *diff*, Dr. Peterson believes. "If you have a fast test and only put people into contact precautions or isolation, which is gowns and gloves, when the test is positive, you isolate many fewer people and you save on the isolation costs. Plus most people don't like to be in contact precautions."

When the hospital started employing the PCR test for C. *diff*, "we had basically a doubling of our C. *diff* rates because of the 95 percent sensitivity of the test. But our Infection Control program has succeeded in getting the rates very, very low, after three years of intense work."

Far more serious than C. *diff* is sepsis, and one of the top three causes of sepsis is the *Candida* fungus, a common source of hospital-acquired infections, says Eleftherios Mylonakis, MD, PhD, of Rhode Island Hospital and professor of medical science and chief of the Division of Infectious Diseases at Warren Alpert Medical School of Brown University. "It's among the three or four most common hospital-acquired causes of bloodstream infections."

Speeding the turnaround time of a candidemia diagnosis is a critical goal, Dr. Mylonakis says. "The sooner you diagnose, the sooner you start appropriate therapy, and the outcomes can be dramatically better. In the case of *Candida* bloodstream infection, the mortality goes from about 40 percent to about half of that."

Dr. Mylonakis

The second important benefit is the ability to withhold or discontinue therapy very early. "What we do with infections that have a high mortality is we tend to over-treat them," says Dr. Mylonakis. "For the two to five days you are treating the patient and it doesn't help, it results in great expense, toxicity, and greater use of antimicrobials which breed resistance."

More and more recent data show that even the delay of two or three hours in appropriate therapy for someone with low blood pressure from a bloodstream infection can affect their mortality, says Dr. Peterson. *Candida* infections are rare at NorthShore, which performs only peripheral stem cell transplants and not solid organ transplants. "They're more common in centers that do bone marrow transplants or liver transplants."

The impact on early turnaround time is a major reason why a novel testing platform developed by the company T2 Biosystems, which has developed a *Candida* test using the platform, is so significant. Dr. Mylonakis led the clinical evaluation of the T2 magnetic resonance platform, T2MR, which found that it represents a breakthrough shift into a new era of molecular diagnostics (*Clin Infect Dis.* 2015;60[6]:892–899).

Unlike microbiology cultures, the T2Candida Panel tests the blood sample directly. "It doesn't depend on the microbes growing on the plate; it works directly on the sample so it takes away the time you need to have the pathogen multiply. This is the main point that differentiates this technology from other tests on the market," Dr. Mylonakis explains.

The T2MR technology works by using the disturbance in the environment surrounding water molecules in a clinical

specimen caused by a clustering of nanoparticles that are super-paramagnetic, says Michael Pfaller, MD, a pathologist and chief medical officer of T2 Biosystems.

“When the sample is pulsed by a magnetic field, it takes a while for the hydrogen molecules to relax back to normal. When the nanoparticles cluster, the extent of the clustering determines the duration of time of the relaxation signal. It’s a novel and unique way of detecting different perturbations in the sample, and depending on what you use for the probes to bind to, that gives you your assay.”

The detection phase in T2MR is the signal amplification as well. Normally, the extraction, enrichment of the target DNA, and cleanup of interfering substances in virtually every molecular technology for whole blood immediately drops the sensitivity to pretty much unacceptable levels for routine diagnosis, Dr. Pfaller notes. “But with the T2MR technology, we have the ability to perform this testing directly from the patient’s sample, which makes it markedly different from anything that’s available right now.”

It’s a technology that could be used for other types of assays, he adds. Right now the company is remaining focused on infectious targets—those pathogens that are not optimally covered by empiric therapy because of their intrinsic or acquired resistance patterns to anti-infectives, where the timing of therapy means saving lives. “Our next three diagnostic applications are called T2Bacteria, T2HemoStat, and T2Lyme, which are focused on bacterial sepsis infections, hemostasis, and Lyme disease, respectively. We plan to initiate clinical trials in the second half of 2015 for T2Bacteria Panel and in 2016 for T2HemoStat,” Dr. Pfaller says.

T2 chose *Candida* to start with because the fungus is much more common and important than most people understand. “First and foremost, the way that we diagnose candidemia and invasive candidiasis by blood culture is suboptimal, and the sensitivity of blood culture is probably in the range of 40 to 50 percent. So we’re missing at least half of serious cases of candidiasis. *Candida* is known to be the No. 1 hospital-associated primary bloodstream infection, and other data show it ranks right up there with *Staphylococcus aureus* as a frequently reported hospital pathogen. It’s a major concern for patients who are immunocompromised or in the ICU.”

“People tend not to want to believe that,” says Dr. Pfaller, who has been studying *Candida* for nearly 30 years. “But it’s the infection we probably do the worst in managing. As to sepsis caused by candidemia, this is a disease where we haven’t seen a change in mortality in at least 25 years. The first dip in mortality was when we saw fluconazole introduced, and now we have even better drugs like the echinocandins, but we also have many more critically ill patients becoming infected with *Candida*.”

Exposure to these two classes of antifungal agents is a major factor in the emergence of resistance in the U.S., where aggressive empiric therapy is much more the rule than in other countries, Dr. Pfaller says.

Dr. Pfaller

Candida largely occurs in patients who have been in the hospital a long time; the median time to diagnosis is about 22 or 23 days in the hospital, with the highest-risk patients in surgical intensive care. “All the risk factors for *Candida* are certainly important risk factors for other kinds of infections. But primarily you have patients who are first on broad-spectrum antibacterial agents, which allow overgrowth of *Candida* in the gut. Then when an opportunity strikes, that’s the organism that gains access to the bloodstream, resulting in sepsis and death.”

For its *Candida* test instrument, which runs about $150,000 with a list price of $265 per test, T2 Biosystems is targeting mostly large tertiary care institutions that see this mix of high-risk patients, plus hospitals that use a lot of empiric antifungal therapy. “Those places generally understand they are not optimally managing those

patients," Dr. Pfaller says. While *Candida* patients don't constitute a large population in the hospital, they are often the most care-intensive and expensive-to-treat population, and economic studies have shown that decreasing mortality and excessive antifungal therapy can make the T2Candida test cost-effective to the institution as a whole.

Such a test could obviate some other testing of *Candida*. "For a long time in our laboratory, we were trying to give the clinicians an idea of whether or not it was *Candida*," Dr. Schuetz says. "Species is important because *Candida* glabrata has higher rates of resistance to fluconazole than some of the other species such as *Candida* albicans. However, it seems that more and more clinicians are using the echinocandins, a broad-spectrum antifungal, at least initially for candidemia, regardless of the *Candida* species." So she has been wondering about the utility of doing a species-specific *Candida* rapid molecular test up front, except with non-immunocompromised patients in whom the treatment might target a narrower spectrum.

Of the T2 Biosystems test, Dr. Schuetz says, "Bringing in something that would diagnose candidemia before the cultures flag positive, to get these patients on appropriate therapy, would be fantastic. A lot of patients who get candidemia could get a lot of other things that non-immunocompromised patients wouldn't be at risk for. So it's always good to know early on if it's candidemia that's making the patient so sick. In some cases, the physician may have a critically ill ICU patient and may not have even been thinking about candidemia."

When the first Chicago area hospital reported a rare outbreak of a very drug-resistant organism, carbapenem-resistant Enterobacteriaceae (CRE), a couple of years ago, Dr. Beavis says, it seemed like an isolated event linked to endoscopes.

"Then this past year, there were outbreaks in several institutions, and we realized this might not be a rare situation and we implemented a protocol." In March, the Centers for Disease Control and Prevention also published protocols that are similar. "They suggest you use a brush to clean scopes and put the brush in broth, and if the broth turns turbid, meaning bacteria are growing, then you put some of the broth on a MacConkey plate and a blood plate to get colonies of these organisms."

The carbapenem-resistant organisms are resistant to just about all antimicrobials we have, Dr. Beavis says. "So if that organism goes from the GI tract into a patient's bloodstream, then we really don't have many effective treatments. Moreover, once that happens, people can become carriers, and the organism can follow the patient from facility to facility. So part of this is you don't want patients to become carriers."

'Every program in the country is certainly looking at possible CRE in their endoscopes.'

Lance Peterson, MD

The protocol her hospital developed in-house involves putting the broth on the plate from the beginning. "This allows us to get the colonies a day sooner and allows for more rapid identification of any Gram-negative organisms that might be present. So because we use a MALDI-TOF to rapidly identify these organisms, we can put a result out within 24 hours of getting the broth."

It's a labor-intensive process, Dr. Beavis says. "In the GI units, they're having to squirt water through each of the channels of the scope, then use a brush to clean it, so when we receive a specimen in the microbiology lab, it's often 200 mLs of fluid with a long brush inside, and we're spinning that down to concentrate any organisms that might be present."

Cutting off 24 hours of turnaround time helps in preventing transmission of the organism, Dr. Beavis notes. “While we’re waiting for the culture, the scopes are in quarantine. And the scopes are very expensive; we don’t want to keep them out of service for three or four days while we’re getting the results back.”

However, she adds, “We’re all starting from scratch. The CDC protocol is interim, and there are going to be differences. Each hospital might have to come up with its own protocol.” Microbiology labs in Chicago will be comparing protocols at their next quarterly meeting. Only time will tell, she says, if the measures they are taking will reduce the incidence of the organisms and the possibility of transmitting them from one person to another.

“Every program in the country is certainly looking at possible CRE in their endoscopes,” says Dr. Peterson, noting that NorthShore has a rigorous disinfection program. “The companies modified the scopes a year or two ago which made cleaning the elevator mechanism difficult, and I suspect that’s where the problem is.”

Multiple different genetic mechanisms can cause CRE; it’s not always the same organisms, Dr. Peterson adds. “For a long time, there was no way to screen for CRE, and it had taken a pretty good foothold in New York in the Brooklyn area,” even moving out of the ICU into the normal patient units. But with surveillance, a lot of the hospitals have been able to reduce their rates.

For the past four years, NorthShore’s molecular diagnostics laboratory has been doing its own laboratory-developed PCR screening for CRE in the ICUs, one of perhaps two such labs in the country. “There are culture-based tests for CRE too, but the sensitivity is only in the 75 to 80 percent range, so you’ll end up missing some. I do think this is a big challenge for labs right now.” But developing a commercial assay will also be challenging because it’s hard to find a lot of patients who have the organism.

The CRE cases are few and far between, Dr. Peterson says. “We get maybe six or eight patients from other hospitals or nursing homes who are admitted here, and we put them in contact precautions so it doesn’t spread in our hospital. We screen all of our ICU patients every month, and if we find someone we screen everyone again.”

The Gram-positive rapid diagnostic tests seem to have made a lot of progress in the past five years, Dr. Peterson says. “There are molecular tests that can rapidly identify coag-negative staph and *Staphylococcus aureus* whether they’re methicillin resistant or not. There are also molecular tests that can identify MRSA and *Staphylococcus aureus* directly out of skin wounds. Those are not inexpensive, but they’re pretty simple assays to do and generally the answer’s available in an hour or two.”

But the Gram-negatives are the big challenge for the microbiology laboratory, in his view. “We’re seeing new Gram-negative resistant mechanisms arising all the time, first the CRE from New York, then the extended-spectrum beta-lactamases starting in South America, a different CRE from India, and so on. You need a test that can pick them up quickly even though you don’t know the genetics, so you can treat the patient correctly, such as a reliable rapid phenotypic susceptibility test.”

Dr. Peterson

“So the big challenge now and for the next four or five years will be the Gram-negative side of things: How do you pick up very quickly a CRE or even an ESBL? Because with many of these drug-resistant organisms, it doesn’t matter what the genetic mechanism is. Many of these resistant mechanisms are a combination of different things going on in the bacterial cell that you can’t readily pick up with even a larger array of genetic tests.”

With S. aureus, most of the roughly 20 clonal complexes can’t tolerate the mecA gene that drives methicillin

resistance, Dr. Peterson says. “So it’s a relatively small genetic pool, whereas for the Gram-negatives there’s all kinds of sequence types or clonal complex types that can pick up resistance mechanisms. We really don’t fully know how to solve that problem.”

But NorthShore has a strong record in controlling infectious disease and is one of the nation’s MRSA containment success stories. It was the first hospital system in North America to start screening everyone for MRSA and putting positives into contact precautions and decolonizing them at the same time, and at the 10-year anniversary of its aggressive program, MRSA is well under control, Dr. Peterson says. “We’re running about two or three infections per 10,000 patients, which is about 10 times lower than most hospitals report. We have almost no bloodstream infections—maybe one or two in all four of our hospitals every year.”

The transmissibility of different hazardous pathogens to laboratory workers is a whole other category of worry, Dr. Schuetz says. On that score she has found herself in frequent discussions with other microbiology laboratory directors about not only Ebola but also Creutzfeldt-Jakob disease.

“We see CJD as a critical pathogen because it’s so important to protect ourselves up front from it, and to try to figure with clinical teams how we can best do this,” Dr. Schuetz says. Of the 1,500 cultures her laboratory does with cerebrospinal fluid each year, one or two a month are to rule out CJD, and they cause the lab to kick into high protective gear.

“Even though the World Health Organization lists CJD as a low infectivity pathogen, it still should be treated with extra precautions, and I think WHO has been a little bit unclear on that. There are particular concerns about transmissibility and exposure to prion disease through exposure to CSF,” Dr. Schuetz says.

The specific protocol for a “14-3-3 order” to test CSF to rule out CJD begins with an immediate stop. “We won’t process the specimen. We call the microbiology pathology resident; then that resident immediately contacts the team to see how high the differential CJD is. If it’s extremely low on the differential, we will still work up the culture, but with extra precautions. If it’s high on the differential, some reference labs would rather not accept the specimen to do viral PCR testing on it until CJD has been ruled out. “

Her laboratory also has to let all the other labs in the hospital know if the specimen is high on the differential. “We let molecular pathology know, and we have to let chemistry and the core labs know in case they’re running tests on it, because there are issues they have with cleaning the instrument and the workspace afterward.” Dr. Schuetz is surprised to hear some labs say they treat CSF from CJD patients the same as anything else. “Having a process in place to work with these types of pathogens is really important.”

Along with C. *diff*, CRE, *Candida*, and other microbes, CJD dramatizes the constant need for microbiology to be prepared to deal with challenging new pathogens. Says Dr. Peterson, “It’s very important for all microbiology laboratories to know their patient population and infectious disease probably on an annual basis, sit down and meet with the leadership of infection control, show them what kinds of infections they’ve had, and talk about what kinds of diagnostic testing, if any, needs to change in order to keep up with the kinds of infections you’re seeing in practice.”

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