

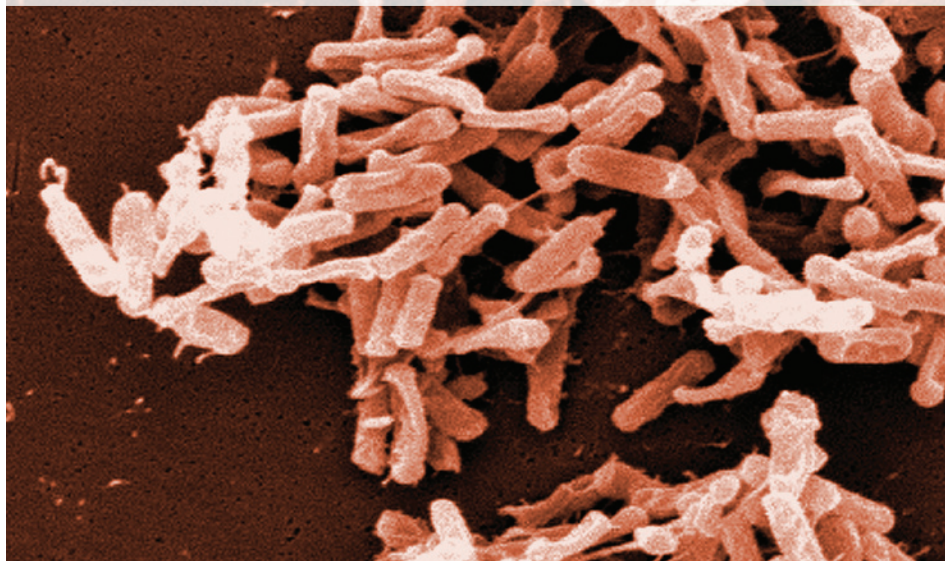
CEPHEID

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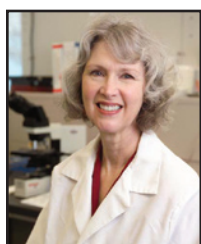
David Persing, M.D., Ph.D.

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Outbreak! The New *Clostridium difficile*

Basic Incidence and Testing Assumptions Called Into Question



Ellen Jo Baron, Ph.D.

Director, Medical Affairs,
Cepheid

Associate Director, Clinical
Microbiology Lab, and Interim
Director, Virology Lab, SUMC

Professor, Dept. of Pathology,
Stanford Medical School

Wake-up Call

On November 11, 2008, the United States got a wake-up call on the extent of the *Clostridium difficile* infection (CDI) problem. The Association for Practitioners in Infection Control and Epidemiology (APIC) announced the results of a one-day prevalence survey conducted between May and August of 2008.¹

The numbers were astounding and sobering. More than 12 of every 1000 inpatients were infected with *C. difficile*. 73% of these patients acquired their disease in the healthcare setting. Approximately 70% of the patients were older than 60 years. 67.6% of all patients had co-morbid conditions and nearly 80% had received antibiotics within the previous 30 days. Only half of the patients surveyed had resolution of their symptoms within seven days, highlighting the serious morbidity of CDI.

The mortality statistics were also frightening. Given a previously determined mortality rate of 4.2%, 301 of the 7,178 inpatients with CDI on any given day in the U.S. will die from

their disease.^{1,2} One major shortcoming of the survey was that 94.4% of the positive results were based on an enzyme immunoassay performed by the laboratory. As will be further discussed below, the point prevalence survey actually underestimated the depth of the problem by at least 50%!

Dr. William Jarvis, formerly of the Centers for Disease Control and Prevention and the first author of the publication, concluded "Given that not all patients with diarrhea

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Executive Editor
David Persing, M.D., Ph.D.

Managing Editors
Jared Tipton
Stripe Demarest

Lead Author
Ellen Jo Baron, Ph.D.

Contributing Author
Fred Tenover, Ph.D.

Production Manager
Gregory Birgfeld

Graphic Design
Bijal Patel

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Cepheid ON-DEMAND Report
1327 Chesapeake Terrace
Sunnyvale, CA 94089

editor@cepheidondemand.com

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From the Editor:

Rising *C. difficile* Rates as Testing Strategies Evolve: Impure Coincidence?



David Persing,
M.D., Ph.D.

Chief Medical and
Technology Officer,
Cepheid

The lead article written by Ellen Jo Baron for this issue of the **On-DEMAND Report**, contains something of a revelation. Starting with the use of culture-based methods for proving the etiology of nosocomial diarrhea, the article chronicles the evolution of testing methods for *C. difficile* over the years.

Culture-based methods, especially when coupled with detection of toxin-producing strains, were invaluable as aids in the epidemiologic correlation of *C. difficile* with nosocomial infection, but ultimately proved to be impractical for the management of patients with suspected *C. difficile* infection. The turnaround time for culture-based tests is several days, with results arriving long after physicians have already made up their minds as to the probability of infection.

Unfortunately, efforts to improve turnaround time have come at the price of accuracy. In the past decade, *C. difficile* immunoassays have essentially overtaken the diagnostics market for *C. difficile*. Most laboratories in the United States now use toxin antigen detection systems, which despite improvements, still show poor correlation with the historical gold standard of toxigenic culture.

According to several recent studies cited in this article, toxin tests vary in sensitivity levels to as low as 33%, making toxin detection a poor surrogate for detection of the infectious agent itself. From a practical standpoint, this means that many, if not most, cases of *C. difficile* are not currently subjected to isolation procedures that could ameliorate transmission of *C. difficile* in the healthcare setting.

Considering the number of patients who serve as unmitigated reservoirs, perhaps it is no coincidence that *C. difficile* rates nationwide have risen at a pace that parallels the

“Culture-based methods... ultimately proved to be impractical for the management of patients with suspected *C. difficile* infection”

introduction of these tests. For laboratorians, this is an opportunity for reflection:

How did we get it so wrong for so long? Over the next few years, there will plenty of opportunities for finger-pointing in the direction of test manufacturers, proficiency testing programs, and ourselves as lab directors. However, with the advent of sensitive and specific molecular diagnostic methods, the good news is that better options for *C. difficile* testing are on the horizon.

David H Persing

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are tested for CDI and that most facilities use enzyme immunoassays with limited sensitivity to detect *C. difficile*, these are minimum estimates of the U.S. health care facility *C. difficile* burden.”

Test data were received from close to 650 hospitals in 47 states, 12.5% of all the hospitals in the United States. Hospitals of all sizes and types participated. They were primarily acute care, but encompassed pediatric, cancer, chronic care, and cardiac specialties, and ranged from public to private to county facilities.

Explaining the Underestimation

Why are the survey results likely to have actually underestimated the true disease burden from *C. difficile*? Over the years, laboratories have moved from performing culture and testing the isolates for toxin production (known as toxigenic culture, the true gold standard for detection of *C. difficile* in feces), to cytotoxin neutralization cell culture assays (the next best method for laboratory diagnosis). From there, the testing progression moved on to enzyme immunoassays (EIAs) for either toxin A or toxins A and B, with or without simultaneous testing for a protein traditionally thought to be present in all *C. difficile* isolates, *i.e.*, glutamate dehydrogenase (GDH).

It had been assumed that the cell culture cytotoxin neutralization test was the most reliable laboratory assay for CDI diagnosis, although technically difficult and slow to generate results (requiring at least overnight incubation for a preliminary result). But most laboratories that had originally set up this technically demanding assay stopped performing the cell culture cytotoxin neutralization assay during the last decade.

Many experts believe this was because the faster EIA tests were so much easier to perform, did not require expertise or cell culture capability, and the results were thought to be adequate for laboratory diagnosis of most cases of CDI. Dr. Jarvis was well

aware that the accuracy of the EIA tests was questionable. His contention is supported by recent publications.

Comparing the Tests

A newly published comparison conducted at Johns Hopkins Hospital showed that a toxin B cell culture cytotoxin neutralization test was only 67% sensitive when compared to toxigenic culture assay (the true gold standard).³ A PCR assay for toxin B gene sequences (*tcdB* gene) was 83.6% sensitive compared with toxigenic culture, but increased to 90.9% when the previous “standard” cell culture cytotoxin B assay was used as the comparison.

The Hopkins group had previously published an influential paper

The EIAs used for 94.4% of the testing performed during the survey will miss as many as 52% of the truly infected or colonized patients.

advocating a two-step approach, with GDH used as a preliminary screening test and more intense testing performed only for samples positive for GDH.⁴ The cell culture cytotoxin neutralization was the gold standard for this study, and the results showed the GDH two-step algorithm to perform well.

Alarming, the commonly used toxin A and B EIA test used by most laboratories, likely the test most used to detect *C. difficile* infection in the APIC point prevalence study, **was only 36% sensitive against a better comparator.**⁴ Pitfalls with the GDH algorithm included the use of two separate types of tests and the delay (up to three days) in turnaround time for positive results. However, the Hopkins workers claimed that they saved more than \$250,000 per year by not testing

all samples for cytotoxin. Although their positive predictive value was only 53%, their negative predictive value was 99.7%.⁴ Other experienced workers questioned that approach and the scientists at Hopkins revisited the diagnosis of CDI in their 2009 paper.³

Dr. Peter Gilligan from the University of North Carolina Medical Center has been studying and writing about *C. difficile* since 1981. He recently evaluated a new lateral flow device incorporating GDH and toxin antigen (both A and B) detection.⁵ Unfortunately, a toxigenic culture was not performed.

Dr. Gilligan reported that a different GDH assay combined with toxin A and B in a lateral-flow design was more sensitive than the one used by most laboratories. The two toxin A and B

assays that Dr. Gilligan evaluated gave sensitivities of only 43% and 59.5% compared with a cell culture cytotoxin neutralization assay. He concluded that GDH positive samples required a confirmatory test and that the current EIA formats, whether lateral flow or solid-phase, were not sensitive enough for that task.⁵

Toxigenic Culture Method

Originally advocated by Dr. Dale Gerding, the toxigenic culture method has been improved in recent years by the use of anaerobic chambers and better agar growth media. The selective medium cycloserine-cefoxitin fructose

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agar, developed at the Wadsworth Veterans Administration hospital during the 1970s (George, et al.)⁶ has been improved with the addition of horse blood and taurocholate, resulting in better recovery of *C. difficile* from fecal cultures.

Only stools that take the shape of the container, which is how we define true diarrhea, should be tested — unless the physician indicates that the patient has toxic megacolon. To increase yield, fecal specimens are treated with either heat shock (heat a 20% suspension of feces in chopped meat carbohydrate broth at 80°C for 15 minutes) or alcohol shock (suspend the feces 50/50 in 95% or absolute ethanol and mix gently at room temperature for one hour) to kill vegetative cells and allow spores to remain viable.

The resulting suspension is then plated onto selective media and incubated anaerobically. Colonies will grow after 24–48 hours. On blood agar, these colonies fluoresce chartreuse and have a very distinctive horse manure smell. Colonies are inoculated into chopped meat carbohydrate broth, which is further incubated for up to 5 days. The supernatant is then tested for cytotoxin using the cell culture cytotoxin neutralization assay or an EIA for toxin B (less effective).⁷

The cell culture cytotoxin neutralization assay uses fecal supernatant, usually diluted 1:100 or 1:200, layered over a monolayer of human or other mammalian cells in culture, similar to cell cultures used for recovering viruses. When present in the feces, cytotoxin B causes cytopathic effect (CPE), i.e., rounding up and sloughing off of cells from the monolayer. If this effect is specifically inhibited by a *C. difficile* toxin B neutralizing antitoxin (commercially available), the test is considered positive for *C. difficile* cytotoxin. CPE begins showing up at 12–18 hours of incubation but laboratories usually



Nancy Cornish, M.D.
Director of Microbiology at Methodist and Childrens' Hospitals, Omaha, NE.

wait for 48 hours before finalizing a negative result.

Quest for Improvement

Dr. Jon Rosenblatt and colleagues at Mayo Clinic suspected that laboratories were missing important *C. difficile* cases using the two-step approach.⁸ They developed an in-house PCR test for the *tcdC* gene, and compared their results to EIAs and GDH detection, using toxigenic culture as the gold standard comparator.

The best performance among any of the four separate EIA assays that they evaluated was 48% sensitivity. But to their surprise, the GDH assay they tested detected only 32% of the toxin-positive *C. difficile* isolates identified by culture, and was only 76% sensitive versus culture for *C. difficile*. The Mayo group concluded that GDH was neither a sensitive alternative to culture nor an accurate screening method for toxin-positive stools.⁸ Even their own PCR for two *C. difficile* genetic targets was only 86% sensitive compared with toxigenic culture.

When the results of the APIC point prevalence study are re-evaluated, taking into consideration that EIA assays which fail to detect as many as 52% of *C. difficile*-infected patients were used to generate 94.4% of the results, it is clear that the actual magnitude of the CDI problem in the U.S. was greatly underestimated.

Speed Counts

The two-step algorithm lacks sensitivity, and as the data cited above show, the delay in results may have disastrous consequences to patients. Dr. Nancy Cornish, Director of Microbiology at Methodist and Childrens' Hospitals, Omaha, Nebraska, has articulated her concern about the state of *C. difficile* testing. Dr. Cornish is a strong voice for pathologists, publishing often on microbiology-related issues in CAP Today, the monthly publication of the College of American Pathologists.

Dr. Cornish said: "When I realized that our physicians were not waiting for our laboratory results when we used the two-day cytotoxicity tissue culture assay test to confirm positive GDH results, but were resorting to performing colonoscopy procedures to confirm the diagnosis of *C. difficile* colitis, then the decision to change testing methods to include PCR was easy. The cost-benefit ratio of having a rapid result that delivers the best possible sensitivity and specificity is very attractive to our clinicians.

"They are interested in having this test as it will allow a rapid diagnosis resulting in faster treatment and implementation of Infection Control measures in addition to reducing the use of unnecessary testing such as colonoscopy. A rapid negative result allows the clinician to pursue testing for other causes, saves the patient from having to take unnecessary antibiotics and saves the hospital the costs of isolation." Currently, the hospital is validating Cepheid's GeneXpert RUO product.

and How it Impacts Hospitals Nationwide

Action Items for Hospitals

What should healthcare institutions be doing to control CDI? Dr. Cliff McDonald from the Centers for Disease Control and Prevention in Atlanta has outlined surveillance strategies for infection control monitoring.⁹ If invasive techniques such as endoscopic evaluation are not used, a positive laboratory test is necessary for the diagnosis of CDI.

institution? First, the offending antibiotic should be discontinued if possible. Second, treatment with metronidazole or vancomycin should be started.¹¹ Vancomycin has been advocated for more severe disease.¹¹

Both medications have been associated with relapses. If retreatment fails, other treatment options are sometimes tried, such as probiotics, immunotherapy, or “fecal transplant.” Aslam and Musher reviewed treatment approaches in

antibiotic usage can contribute to its persistence. Does knowing that NAP1 is present in your hospital translate into any changes in practice?

Dr. Dale Gerding, in a 2007 editorial, suggested that knowledge of whether the infecting strain were the hypervirulent NAP1, 027 strain could be important, at least epidemiologically.¹⁴ Patients are immediately placed on contact precautions, as the spores persist in the environment and spread the pathogenic strain from patient to patient. Healthcare personnel must forego alcohol gel hand disinfection and go back to soap and water, the only effective handwashing method when spores are in the environment.

For each false-negative laboratory test, patients are denied appropriate therapy and infection control practices are delayed.¹⁴ At the time of the editorial, Dr. Gerding was correct that only toxigenic culture methods were able to detect pathogenic *C. difficile* with sufficient sensitivity to be reliable, and that culture was the only test that would yield the epidemiologic and treatment information of presence of the NAP1 hypervirulent strain. However, the results were only available days after the initial patients present with symptoms, thus of primarily epidemiologic interest.

More institutional systemic activities include terminal disinfection of any room in which a CDI patient was housed. The anthrax attacks of 2001 created the need to disinfect entire buildings containing spores. Some of the techniques developed then are being evaluated for patient room disinfection in cases of CDI.

Boyce and colleagues have reported that hydrogen peroxide vapor decontamination, although more difficult to administer than cleaning with bleach, was successful in eliminating spore recovery in vapor-treated rooms and that the incidence of *C. difficile* infection dropped

“...a rapid result that delivers the best possible sensitivity and specificity, and can also detect the more virulent 027/NAP1 strain is very attractive...”

— Dr. Nancy Cornish, Director of Microbiology at Methodist and Childrens' Hospitals, Omaha, NE

In addition to healthcare-associated disease, Dr. McDonald advocates for surveillance for community-acquired disease. In a 2008 study conducted among six hospitals in North Carolina, Kutty and coworkers found that 58% of 1,046 CDI cases identified were community onset. Of those, 34% had no healthcare institution exposure.¹⁰ Clearly, the algorithm for testing patients with diarrhea is changing rapidly. Laboratories that previously only tested inpatients after the third day of their admission should be modifying their practices.

Benefits of Fast, Reliable Identification

What activities does a rapid and reliable test result for the presence of *C. difficile* in a patient's stool generate in the healthcare

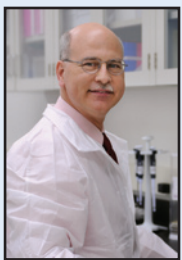
2006.¹² Some experimental agents such as nitazoxanide and oritavancin are still under investigation.

The Advent of NAP1

The rise in rates and severity of CDI in the last several years has been paralleled by an increase in the detection of a recently recognized strain of *C. difficile*, called variously North American Pulsed Field Gel type 1 (NAP1), ribotype 027, or BI (based on restriction enzyme analysis).¹³ This strain may cause more severe disease than other strains because of its increased production of toxin B, the major virulence factor of *C. difficile*. The NAP1 strain also produces more spores, purported to give it an environmental advantage. Importantly, this strain is quinolone resistant, and

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VRE—Still Here, Still a Problem



Fred C. Tenover,
Ph.D., D(ABMM)

Senior Director,
Scientific Affairs

Vancomycin-resistant enterococci (VRE), could be considered the “Rodney Dangerfields” of the microbial world. They often get little respect from infection preventionists or physicians even though enterococci overall are the third most common

vanB, or *vanD* genes) and exclude the intrinsically, *vanC*-mediated, vancomycin-resistant species of *Enterococcus gallinarum* and *Enterococcus casseliflavus*, which demonstrate low-level resistance (vancomycin MICs usually in the 4–16 µg/mL range). Other vancomycin resistance genes (e.g., *vanE* and *vanG*) remain very rare among enterococci and have not been associated with outbreaks of disease.³

Grounds for Concern

Enterococci are the second most common cause of central-line associated bloodstream infections,

Patients were offered a potential reprieve from the scourge of VRE with the approval of quinupristin-dalfopristin, linezolid, and daptomycin in the late 1990s and early 2000s. These drugs proved to be effective for treatment of enterococcal infections, although side effects, particularly with quinupristin-dalfopristin, were commonplace.

The reprieve was short-lived. Even in 2007, VRE were already showing signs of developing resistance to linezolid and other antimicrobial agents, making multi-drug resistant VRE a therapeutic and infection control issue once again.⁴

Estimating Incidence

Unfortunately, there are no national surveillance data on overall number of VRE infections in U.S. hospitals. However, Reik et al. used national survey data from hospital discharges in conjunction with national antimicrobial resistance survey data to make estimates about the number of enterococcal and VRE infections nationwide.⁵ Because of the inexact nature of hospital coding, Reik and colleagues made both conservative and liberal estimates using Group D streptococcal codes (i.e., the historical designation for organisms now called “*Enterococcus* species”), versus those codes plus a percentage of infections at various body sites (i.e., blood, urine, and wounds) based on an extensive literature review.

These investigators estimated conservatively, based on ICD-9 patient diagnosis codes, that there were 20,931 VRE infections out of a total of 125,134 enterococcal infections in 2004 in U.S. hospitals. By adding on percentages of blood, urinary tract, and wound infections, in addition to the numbers of Group

“Enterococci are the second most common cause of central line associated bloodstream infections...”

cause of healthcare-associated infections, according to the 2006–2007 National Healthcare Safety Network (NHSN) data.¹

VRE including isolates of *Enterococcus faecium*, *Enterococcus faecalis*, and occasional other species of enterococci, for which the vancomycin minimum inhibitory concentrations (MICs) are less than 32 µg/mL,² continue to spread and cause outbreaks among hospitalized patients throughout the world.

The strains of VRE that should be considered for infection control interventions are those with acquired vancomycin resistance (predominantly involving *vanA*,

the third most common cause of urinary tract infections, and the third most common cause of surgical site infections and device-associated infections. In fact, the number of VRE device-associated infections is equal to the number of device-associated methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

When VRE became a concern for clinicians and infection preventionists in the 1990s, it was primarily because the strains were untreatable. Clinicians had to resort to giving continuous infusions of antimicrobial agents, trying to identify potential synergism with combinations of less active agents, and a number of other creative approaches when confronted with serious VRE infections.

D streptococci reported, the liberal estimate jumped to 85,586 VRE infections out of a total 521,285 enterococcal infections — a fourfold increase in the apparent incidence.

Considerations for Control

Why is it that some hospitals put considerable effort into controlling VRE and others do not? One reason is the nature of patients in the institution. Bone marrow and stem cell transplant patients are at high risk for colonization and infection with antimicrobial-resistant pathogens, and particularly with VRE. Thus transplant centers are especially concerned.

Calderwood and colleagues suggested that 30% of transplant patients who are colonized with VRE will develop overt infection. Thus, active surveillance for VRE in this patient population has particular value.⁶ Huang et al. also studied enterococcal carriage through an active surveillance program using bacterial culture techniques. Surveillance cultures to detect VRE were performed on patients on admission and weekly thereafter while they were inpatients.

Results of the cultures increased the identification of VRE-colonized patients by 2.2–17.0 -fold on admission and by 3.3–15.4 -fold in the subsequent weeks in comparison with the identification of colonized or infected patients only through routine cultures ordered for diagnostic purposes.⁷

VRE colonization and infection is also an issue for pediatric patients. Milstone and colleagues reported that the use of weekly surveillance cultures increased the number of VRE carriers detected in their pediatric intensive care unit by 350%.⁸


Screening Challenges

Screening for VRE is not as easy as screening for MRSA. Unfortunately, the *vanA* and *vanB* vancomycin resistance genes are usually located on transmissible plasmids or conjugal transposons, *i.e.*, mobile genetic elements that move among several species of enterococci, and, in the case of *vanB*, can also be present in the anaerobic flora that inhabit the human gastrointestinal tract.

Molecular tests to detect the *vanA* and *vanB* vancomycin resistance genes

don't specifically link those genes to an enterococcal host bacterium the way the *mecA* gene is linked to the *S. aureus* chromosome (*i.e.*, via *orfX*, a genetic linker sequence) in MRSA.

Several studies have shown that detection of *vanA* is highly associated with recovery of a *vanA*-containing *Enterococcus* species from stool.^{9, 10} However, the same cannot be said for *vanB* genes, which are widely distributed among anaerobic bacteria.^{11–14} Interestingly, according to Australian researchers, the *vanB* gene in one strain of *Clostridium symbiosum* was located on a mobile genetic element that was transferable to both *E. faecium* and *E. faecalis* in the digestive tracts of germ-free mice.¹⁵

Does finding a transmissible vancomycin resistance gene outside of enterococci diminish or actually enhance the value of the *vanB* test? Given the possibility of transfer of vancomycin resistance to a co-colonizing gut *Enterococcus* in a time of increasing enterococcal resistance to the few effective antibiotics left for treating this microbe, the debate takes on new urgency. 

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significantly during the intervention period.¹⁵ This is even more remarkable when one considers that the outbreak in Boyce's institution was caused by the NAP1 increased spore-producing strain. Knowing that this strain was present in a healthcare environment could trigger the more effective but more difficult to administer peroxide vapor disinfection.


Finally, antibiotic restriction has also been effective in reducing CDI incidence in facilities with epidemic

situations.¹⁶ The outbreak strain in the case reported by Kallen and colleagues at the CDC was also NAP1.¹⁶ In addition to fluoroquinolone restriction, which significantly reduced cases of CDI at the community hospital studied, the hospital also changed its environmental services provider, ostensibly resulting in more effective cleaning strategies.

In summary, the way we diagnose *C. difficile* infection is about to change. Factors contributing to this paradigm shift include:

- Advent of a hypervirulent new strain

- Realization that CDI is more rampant than previously imagined
- Recognition that current widespread testing methods are inaccurate or too slow to benefit patients and infection control practices
- Availability of rapid and specific molecular tests, placing the ability to obtain the correct answer in less than an hour in the hands of every laboratorian

Once clinicians and infection preventionists know what they are dealing with, they can quickly move to intervene. 



1327 Chesapeake Terrace
Sunnyvale, CA 94089
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Highlights from Previous Issues:

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Volume 2, Issue 1, April 2009

Celebration of the 30th Anniversary of *Clostridium difficile*
Volume 1, Issue 2, September 2008

Testing Patients for MRSA and *Staph aureus* Before Inpatient Surgery
Volume 1, Issue 2, September 2008

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