DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION
FOOD AND DRUG ADMINISTRATION
NATIONAL INSTITUTES OF HEALTH

Emerging Clostridial Disease Workshop
May 11, 2006
Atlanta, Georgia

Summary of Proceedings

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ATTACHMENT 2

Acronyms Used In This Report

B. subtilis	—	*Bacillus subtilis*
CA	—	Community-Associated
CDAD	—	*C. Difficile*-Associated Disease
CDC	—	Centers for Disease Control and Prevention
C. difficile	—	*Clostridium difficile*
CO	—	Community-Onset
C. sordellii	—	*Clostridium sordellii*
EIP	—	Emerging Infections Program
FDA	—	Food and Drug Administration
GI	—	Gastrointestinal
GM-CSF	—	Granulocyte Macrophage-Colony Stimulating Factor
HA	—	Healthcare-Associated
IDSOG	—	Infectious Disease Society of Obstetrics and Gynecology
LPS	—	Lipopolysaccharide
LTCF	—	Long-Term Care Facility
MIAs	—	Medically-Induced Abortions
MMWR	—	*Morbidity and Mortality Weekly Report*
NIH	—	National Institutes of Health
NNDS	—	Nationally Notifiable Disease System
OB/GYN	—	Obstetrical/Gynecological
PCR	—	Polymerase Chain Reaction
PPIs	—	Proton Pump Inhibitors
TSS	—	Toxic Shock Syndrome
VA	—	Veterans Affairs
WBC	—	White Blood Cell

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EMERGING CLOSTRIDIAL DISEASE WORKSHOP
May 11, 2006
Atlanta, Georgia

Workshop Report

The Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and National Institutes of Health (NIH) convened the Emerging Clostridial Disease Workshop. The proceedings were held on May 11, 2006 at CDC's Global Communications Center in Atlanta, Georgia. The list of participants is appended to the report as Attachment 1.

The goal of the public workshop was to identify research needs and priorities to enable rapid progress in understanding the virulence, pathogenesis, host factors and non-antimicrobial risk factors contributing to reports of morbidity and mortality associated with *Clostridium difficile* (*C. difficile*) and *Clostridium sordellii* (*C. sordellii*). The workshop would also result in a draft research agenda with recommendations for detecting cases and conducting surveillance of diseases and organisms.

Opening Session

Dr. Rima Khabbaz is the Director of the CDC National Center for Infectious Diseases. She welcomed the participants to the workshop and emphasized that CDC hopes the workshop will assist in focusing research and prevention efforts related to *C. difficile* and *C. sordellii*. CDC is committed to strengthening its knowledge on the tremendous morbidity, significant mortality and increased disease severity associated with these serious and challenging infections. Dr. Khabbaz confirmed that CDC will respond to the input, recommendations and next steps provided during the workshop on research and surveillance to address *C. difficile* and *C. sordellii*.

Dr. Paul Seligman is the Associate Director for Safety, Policy and Communication in the FDA Center for Drug Evaluation and Research. He also welcomed the participants to the workshop and explained that reports have been emerging in the United States over the past few years on serious *C. difficile* and *C. sordellii* infections. *C. difficile* is a toxin-produced anaerobe that primarily occurs with antibiotic use and is generally manageable with established therapy. *C. sordellii* was believed to rarely produce toxins or clinical illness.

In 2005, however, CDC published reports of unusual patterns and characteristics of *C. difficile* and also published a case series of deaths from *C. sordellii*. These clusters raised important public health questions about *Clostridium*'s microbiologic ecology and its relationship to fatal illnesses and pregnancy. CDC, FDA and NIH acknowledge the critical need to vigorously approach these issues with scientific rigor, conduct research, and perform surveillance to mitigate public health effects of *C. difficile* and *C. sordellii*.

Dr. Seligman described individual and collective actions CDC, FDA and NIH will take to fill data gaps in *C. difficile* and *C. sordellii*. Jointly, the agencies will thoroughly review and discuss input and recommendations provided during the workshop as well as additional information submitted to the public docket. Each agency will post the workshop transcript, summary and materials submitted to the docket on its respective web site for public access and review. The agencies will produce a draft research agenda with recommendations for detecting cases, conducting surveillance, and enhancing knowledge of the virulence, pathogenesis and treatments for *C. difficile* and *C. sordellii*. The agencies will publish proceedings of the workshop in a peer-reviewed medical journal.

Individually, CDC will closely analyze additional types of monitoring for healthcare-associated (HA) *C. difficile* infections and emerging community-associated (CA) infections. New and evolving risk factors for these diseases will be defined. Definitions and strategies will be developed to better monitor *C. sordellii* infections.

FDA will establish realistic time-lines for obtaining more knowledge on the pathophysiology and etiology of *C. difficile* and *C. sordellii* and determining whether regulatory action affecting the appropriate use and availability of these drug products is warranted. NIH will continue to lead research on the pathogenesis and biology of emerging infections. Efforts will be made to encourage the research and product development communities to address *C. difficile* and *C. sordellii* in future research proposals.

PANEL 1-SESSION 1: CLINICAL SYNDROMES, PATHOPHYSIOLOGY AND HOST FACTORS OF *C. DIFFICILE*

The Panel 1-Session 1 speakers made presentations to define the current emerging clinical syndrome, knowledge gaps and recommendations for basic, applied and clinical research for *C. difficile*. The four Panel 1-Session 1 presentations are outlined below.

Clinical Aspects of *C. Difficile*-Associated Disease (CDAD)

Dr. Dale Gerding is the Associate Chief of Staff for Research and Development at the Hines Veterans Affairs (VA) Hospital. He covered the following areas in his presentation. CDAD is acquired by ingestion of *C. difficile* spores. Prior antibiotic exposure places patients at risk for CDAD. Diarrhea that is mediated by toxins is the most common symptom of *C. difficile*, but severe colitis, sepsis and death may also result. FDA has not approved the use of metronidazole, rifaximin or nitazoxanide for treatment of CDAD.

The current hypothesis is that CDAD occurs usually among hospitalized patients who have taken antibiotics and acquired a toxigenic strain of *C. difficile* but fail to mount an anamnestic IgG antibody response to toxin A. The four major clinical problems with the disease are (1) an inability to prevent CDAD in hospitals and other high-risk settings; (2) the lack of a sensitive and rapid diagnostic test for CDAD; (3) the absence of a treatment to prevent recurrence of CDAD; and (4) an inability to effectively treat fulminant CDAD.

Several general principles are applied to diagnose CDAD. Stool culture is the most sensitive test for CDAD; the cell cytotoxin assay is the most specific test; and the enzyme immunoassay for toxins A and B is the most widely used test to diagnose CDAD based on a survey that was recently administered to infection control and disease physicians, but is only about 70% as sensitive as culture. Flexible sigmoidoscopy is rapid, but only has a 50% sensitivity rate in detecting pseudomembranes. A rapidly rising white blood cell (WBC) count is a clue to fulminant CDAD.

In terms of current options for the treatment of CDAD, metronidazole and vancomycin are antimicrobial drugs that disrupt the flora and leave ~20% of patients susceptible to recurrence, relapse with the same organism or re-infection with a new organism. Newer

treatment possibilities include very narrow spectrum antimicrobial drugs, toxin-binding agents, biological agents that prevent re-colonization, and active and passive vaccines.

A paper published in 2006 on the treatment of the first recurrence of CDAD showed the CDAD recurrence rate was correlated with age and length of hospital stay. Metronidazole was not found to be inferior to vancomycin for treatment of the first recurrence of CDAD. Treatment of the first recurrence of CDAD with the same or a different agent made no difference in outcomes. Complications of shock, colectomy, perforation, megacolon or death were seen in 11% of patients with the first recurrence. This rate was higher than previously observed and is a caution to institute treatment of recurrences rapidly.

A paper published in 2002 on multiple recurrences of CDAD showed the risk of a subsequent CDAD episode in patients with a previous recurrence was 45%. New *C. difficile* organisms caused 50% of recurrences. Anecdotal support was demonstrated for many empiric treatments, such as vancomycin regimens, biotherapeutic approaches, passive treatment with immunoglobulin, toxin-binding agents, and fecal reconstitution using espousal donors, however, no adequate randomized controlled trials are available.

New clinical issues are emerging for *C. difficile*. CDAD rates are increasing and a common epidemic strain was detected in the United States, Canada and Europe. More severe CDAD is being observed with higher rates of mortality and colectomy. The efficacy of metronidazole treatment is now being questioned. Disease in the community and peripartum cases may be increasing. The use of proton pump inhibitors (PPIs) may increase the risk for CDAD. Discharge data show that CDAD rates doubled in VA hospitals from 2000-2004 and similar rate increases are occurring in all US hospitals.

A additional toxin known as binary toxin, variations in the *tcdC* gene and high-level resistance to fluoroquinolone antibiotics have been identified as three characteristic virulence factors of new strains. Fulminant CDAD appears to be increasing. Patients with fulminant disease may exhibit toxic megacolon, hypotension, sepsis, ileus or perforation. A rapid rise in WBC counts may be a clinical clue. Delivery of antibiotics to the site of infection may be difficult. Surgical removal of the colon may be life-saving. Controlled trials to better manage patients and an improved clinical algorithm to decide on surgery are critical needs at this time.

Three recent publications have raised controversies about the continued efficacy of metronidazole in treating CDAD. All three papers showed a higher failure rate of the drug in treating CDAD. Response rates decreased from ~95% to ~75%, while

recurrence rates increased from ~20% to ~30%. CDC received voluntary reports from four states over a 28-month period of 23 CA-CDAD cases and 10 peripartum CDAD cases. Of the CA-CDAD cases, 24% reported no antibiotic use in the previous three months. Of the two isolates recovered, both had the binary toxin gene, one had the *tcdC* gene deletion, and neither was the new epidemic strain. Of four particularly severe peripartum CDAD cases among pregnant women 20-31 years of age, three died and one underwent colectomy.

Data from a U.K. general practice database were published in 2005 and showed alarming increasing rates of CA-CDAD. The rate of CA-CDAD increased from 1/100,000 to 22/100,000 in a ten-year period. The data showed a significant but far lower rate of antibiotic exposure than expected, significant rates of exposure to PPIs other antacid drugs and significant exposure to non-steroidal anti-inflammatory drugs. All of these new and emerging clinical issues emphasize the critical need for stronger research and strategies to prevent and treat CDAD.

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Pathogenesis and Host Response of *C. Difficile*

Dr. Ciaran Kelly is the Associate Professor of Medicine at Harvard Medical School. He covered the following areas in his presentation. Colonic luminal concentrations and colonization resistance influence antibiotic susceptibility of *C. difficile* isolates. Antibiotic therapy disrupts colonization resistance of colonic microflora, but this area warrants further study. Research is also needed to identify antibiotics that are effective in treating *C. difficile*, but do not cause critical changes to the colonic microflora.

Ampicillin, amoxicillin, cephalosporins, clindamycin and quinolones are the antimicrobial agents that most frequently induce CDAD and colitis. However, patients may develop CDAD through cytotoxic chemotherapy, colon preparation or inflammatory bowel disease without antibiotic use. Non-antibiotic-associated CDAD is rare in hospital settings, but may be more common in the community. Two published studies showed that only 36% and 65%, respectively, of CA *C. difficile* patients had a documented history of antibiotic use.

Exposure to *C. difficile* is more likely to occur in healthcare institutions. A high number of spores are present in these facilities and both the environment and personnel can act as sources of *C. difficile*. A published study demonstrated the ability to culture *C. difficile* from several hospital locations and staff. Another study was conducted on the prevalence and acquisition of *C. difficile* among ~300 patients on antibiotics in a high-

risk medical ward with a stay of >2 days. The study showed that 31% of the cohort was colonized at some point during the hospital stay. However, ~50% of colonized patients will not develop disease, while the remaining 50% will develop CDAD.

Toxin A was historically believed to be more important than toxin B due to its enterotoxic effect in animals. However, recent human studies and clinical observations have called this traditional theory into question. Data show that toxin B has an equal or greater injurious effect to the colon than toxin A in colonic explants and intestinal xenographs. Moreover, a small number of toxin A-negative/toxin B-positive strains of *C. difficile* were found to cause significant disease in humans, including severe pseudomembranous colitis. Both toxins A and B cause diarrhea and colitis. These data suggest that both toxins A and B are important in causing disease and need to be addressed by new agents.

In addition to antibiotic use, age, co-morbidity, and innate and adaptive immune responses also serve as host factors to CDAD. Further research on the role of corticosteroids and the innate immune response in fulminant CDAD should be explored. The relationship between the adaptive immune response and CDAD also warrants further study because ~60% of humans have serum IgG and colonic IgA antitoxin antibody. The current hypothesis is that the host immune response plays a pivotal role in determining the clinical outcome of infection with toxigenic *C. difficile*.

A study published in 2000 demonstrated high serum IgG antitoxin A levels in asymptomatic carriers of *C. difficile*. Another study published in 2001 showed high serum IgG antitoxin A levels were associated with a lower risk for recurrent CDAD. These data suggest that a primary immune response during a *C. difficile* episode appeared to be associated with protection against recurrence.

Recent data and increased knowledge of the pathogenesis of CDAD provide opportunities to intervene at different points in the progression of disease. The control of antibiotic therapy and prevention of exposure on colonization will continue to serve as important interventions. A memory response accounts for asymptomatic carriers and a primary immune response assists in protecting against recurrence. Newer antibiotics that are now being studied show promise in terms of having a narrower spectrum of activity and a smaller potential to be associated with recurrence.

The targeted probiotic approach of using a non-toxigenic strain of *C. difficile* to purposely infect the patient and prevent infection with a toxigenic strain is worthy of additional study. Data from the current Phase III tolevamer trial demonstrate promise as a non-antibiotic strategy for treatment of *C. difficile*. A toxoid-based vaccine is

currently in Phase II studies. Several approaches for passive immunotherapy are being considered as mechanisms to protect high-risk persons, such as intravenous immunoglobulins, monoclonal antibodies against toxins A and B, and production of a hyper-immune globulin from vaccinated individuals who undergo plasmapheresis.

Regulation of *C. Difficile* Toxin Gene Expression

Dr. Abraham Sonenshein, of the Tufts University School of Medicine, covered the following areas in his presentation. The relationship between *C. difficile* sporulation and pathogenesis produces three major outcomes. Spores act as a reservoir of disease-causing organisms. Germination in the gastrointestinal (GI) tract is essential for pathogenesis. Toxins A and B are only synthesized during sporulation or the stationary phase. *C. difficile* would be unable to produce toxin, sporulate in the gut or spread disease if the organism had no ability to germinate in the gut. Cells can only sporulate in response to a nutritional limitation.

The onset of sporulation is accompanied by other adaptive responses, such as motility and chemotaxis, secretion of degradative enzymes, transport of secondary nutrients, intracellular catabolic pathways, genetic competence, and antibiotic and toxin production. Spo0A~P is a major transcription factor for early sporulation genes, while RNA polymerase sigma factors, dissociable subunits that direct RNA polymerase to specific promoter sites, turn on large groups of genes at specific times and in specific compartments of the spoulating cell. All sporulation-specific sigma factors are recognizable in *C. difficile*. *TcdR* is a sigma factor for toxin gene transcription that is used for stressful conditions and the control of toxin synthesis.

The C. difficile pathogenicity locus includes five genes. In addition to *tcdB* and *tcdA* genes, which encode two toxin proteins, the *tcdE* gene is suspected of encoding a holing- like protein. This protein may be responsible for the release of toxins into the environment. The *tcdR* upstream gene is believed to act as regulatory protein. Recent studies demonstrate that *tcdR* protein is one of the alternative sigma factors for RNA polymerase. The protein interacts with the RNA polymerase core to direct the enzyme to specific promoter sites for tcdA, tcdB and the *tcdR* gene.

The *tcdR* gene controls toxin synthesis in *C. difficile* and is also closely related to genes that control toxin synthesis or bacteriocin production in *C. botulinum*, *C. tetani* and *C. perfringens*. A previous study demonstrated that the timing of toxin synthesis at protein and messenger RNA levels occurred at the end of rapid exponential growth. Spore formation is not believed to be essential for toxin synthesis, but a clear regulatory

connection exists between these two processes. No expression of toxin genes was found at any time during the growth cycle in the presence of glucose in the medium.

Three physiological issues must be addressed to better understand the regulation of *C. difficile* toxin gene expression: (1) the metabolic signal for nutrient deprivation; (2) the regulatory protein that senses the metabolic signal; and (3) the controlling mechanism between regulatory protein and expression of toxin genes. Previous studies on *Bacillus subtilis* (*B. subtilis*) were used as a model to try to understand this regulation.

The B. subtilis CodY protein serves as a repressor of hundreds of genes that are normally turned on as cells experience nutrient limitation. CodY homologs are nearly ubiquitous in low G+C gram-positive bacteria. CodY recognizes the GTP nucleotide and isoleucine amino acid or valine simultaneously in the cell. *B. subtilis* was used as a surrogate organism due to the inability to produce a CodY mutation in *C. difficile*. The study was designed to determine whether inactivation of a codY gene in *B. subtilis* would affect the expression of *C. difficile* toxin genes.

The deletion of the codY gene in *B. subtilis* resulted in greatly enhanced synthesis of expression of a toxin gene. This finding suggested that the *Clostridium* system was under the control of CodY for *B. subtilis*, but the experiment has not yet been replicated in *C. difficile*. An in vitro experiment demonstrated that the CodY protein of *C. difficile* depends on both GTP and branched chain amino acids to tightly bind to DNA. The tightest site of binding was found to be between two putative promoters that drive expression of the tcdR gene.

The precise molecular mechanism is still unknown, but a model can be constructed for the expression of the *tcdR* gene. A related study showed that the tcdC gene encodes an anti-sigma factor blocking transcription of the A and B genes. A more refined model postulates that CodY protein acts as a negative regulator of the tcdR gene during rapid exponential growth and simultaneously stimulates transcription of the tcdC gene. When cells are in the stationary phase, CodY loses its ability to bind to DNA and stimulate tcdC gene expression. In the absence of a genetic system in *C. difficile*, however, this model cannot yet be confirmed.

Toxins of *C. Difficile*

Dr. Jimmy Ballard is an Associate Professor in the Department of Microbiology and Immunology at the Oklahoma Health Sciences Center. He covered the following areas in his presentation. From 1938 to the present, tremendous advancements have been

made in the study of *C. difficile* toxins, including the initial discovery and analysis, preliminary understanding of cellular activities, additional knowledge in the enzymatic mechanism of action, and the current focus on the atomic level.

Toxins A and B are large clostridial and intracellular bacterial toxins in the type A subfamily of glucosyltransferases. The toxins are found in nearly all clinically-relevant isolates. Immunity to the toxins provides protection from CDAD. The toxins can disrupt cellular physiology; hydrolyze UDP-glucose; terminate signaling through proteins; and change cellular morphology, modulation of the mitochondria and important transcriptional impacts.

Rho, Rac and Cdc42 are small GTP-binding proteins that regulate various events and act as targets to toxins A and B. Studies have shown that other targets may also be important for intoxication, but additional research needs to be conducted in this area. Toxin A results in several effects at the *in vitro* or cellular level, including cell rounding, caspase activation, modulation of the mitochondria, mitogen-activated protein kinase activation, apoptosis and cell death, and increased permeability of epithelial barriers. At the *in vivo* or host level, the effects of toxin A are lethal with fluid accumulation in rabbit ileal loops, neutrophil recruitment, mast cells and macrophages, reactivation of oxygen intermediates, chemokines, cytokine production and neuronal impacts.

At the cellular level, toxin B intoxicates a wide variety of cell types and causes cell rounding, modulation of intracellular signaling pathways, caspase activation, disruption of tight junctions and numerous other impacts related to Rho proteins. At the host level, toxin B is lethal and may also cause systemic and enterotoxic effects. Many of the observed effects of toxin B may be directly attributed to the enzymatic domain.

Studies are underway focusing on the receptor binding domains of toxins A and B. The receptor binding domain can provide protection against the toxin in animal models and may have the ability to engage multiple receptors when interacting with target cells. However, additional research is needed because at least 24 different toxinotypes of *C. difficile* have been identified.

In addition to toxins A and B, *C. difficile* also produces a binary toxin with an A polypeptide that is enzymatic, functions as an ADP-ribosyltransferase and modifies actin in the cell. The binary toxin can be found in 6%-12% of isolates and is associated with recent epidemic strains of *C. difficile*. The binary toxin may also play an important role in colonization, but this area is poorly understood at this time.

The five members of large clostridial toxins are in the *C. difficile*, *C. novyi* and *C. sordellii* species with various levels of lethality. Studies were previously conducted to determine the frequency by which *C. sordellii* produces lethal toxin. An analysis of isolates from cadaver-derived tissue showed that only one produced *tcsL*, all produced a neuraminidase, and a few produced a phospholipase. The isolates also produced a cholesterol-dependent cytolsyin that is found in many *Clostridia* species.

CDC provided 25 clinical isolates of *C. sordellii* for the study, but not all of the isolates produced lethal toxin and many did not produce any detectable cytotoxic factor. Additional research is warranted in this area because these findings are inconsistent with the standard paradigm for *Clostridia*. Most notably, *Clostridia* are known to produce very strong and potent toxins that rapidly cause serious disease.

Several issues will need to be addressed in future studies to strengthen knowledge about *C. difficile* toxins, including (1) toxin B's specific *in vivo* effects and contribution to CDAD; (2) the impact of increased expression of toxins A and B during disease; (3) the overall profile of substrate targets in mammalian cells; (4) specific receptors for toxins A and B; (5) the role of the binary toxin in disease; (6) the contribution of *tcsL* and other toxins to disease; and (7) the mechanism of pathogenesis in isolates that do not appear to produce enterotoxins.

Panel 1-Session 1 Discussion

The Panel 1-Session 1 presenters provided additional details about the clinical syndromes, pathophysiology and host factors of *C. difficile* in response to questions and comments from the participants.

- The lack of selectable markers, methods for simple transformation and recombination are some of the issues that increase the difficulty in advancing genetic manipulation techniques for *C. difficile* and *C. sordellii*. Research is needed to produce a viable genetic system and answer fundamental questions about *C. difficile*. Technologies that might be applied include broad-host-range plasmids, gram-positive conjugated transposon Tn916, suicide plasmids and antisense RNA. However, more progress in genetics is expected to be made with *C. sordellii* because this organism is more sensitive than *C. difficile* to a variety of selectable markers.
- The antibody response against *C. difficile* is typically acquired by 2-3 years of age. Neonates have an extremely high colonization rate and a

very low disease rate. Carriage of *C. difficile* in the GI tract is common in infants, but much lower in adults. Some research suggests that neonates and young infants do not express toxin receptors. Specific elements that make older persons more susceptible to *C. difficile* have not been identified at this time.

- Persons typically do not have high levels of colonization with *C. difficile* upon hospital admission, but patients generally acquire the organism at a rate of ~8% the longer the hospital stay. Data suggest that colonized persons who do not become ill are in a protected state.
- Minimal studies in humans have been conducted on colonization with *C. sordellii*. The published literature contains a paucity of stool or vaginal flora surveys for *C. sordellii*.
- Adherence factors of *C. difficile* are important, but have not been extensively studied. However, some research has shown that altering the microflora of the gut changes the levels and types of expression of glycans on the surface of the epithelium. This process could change binding sites and cause antibiotics to facilitate *C. difficile* colonization. Another study has described a putative adhesion factor in *C. difficile* surface layer proteins that may act as adhesives. Other data indicate that antibody against surface layer protein A has a statistically significant protective effect.
- The association between age and fasting achlorhydria should be analyzed in the context of gastrin levels or pepsinogen in serum. Additional research should also be performed on the role of the Charleson Index and other measures of global co-morbidity in *C. difficile*.
- Previous studies demonstrated that environmental contamination and carriers play an important role in spreading CDAD in hospitals. The data showed that 50% of sites in rooms of patients with diarrhea are contaminated with spores. Spores that are not physically removed or killed with bleach or hydrogen peroxide solutions can remain in the environment for several months. An overwhelming speculation is that contaminated hands of healthcare workers lead to the spread of spores to susceptible patients.
- Environmental sources of antimicrobial agents may perturb host-microbe relationships and disturb niches in the gut. Certain diets or food additives may predispose persons to *C. difficile*.

PANEL 1-SESSION 2: CLINICAL SYNDROMES, PATHOPHYSIOLOGY AND HOST FACTORS OF *C. SORDELLII*

The Panel 1-Session 2 speakers made presentations to define the current emerging clinical syndrome, knowledge gaps and recommendations for basic, applied and clinical research for *C. sordellii*. The five Panel 1-Session 2 presentations are outlined below.

C. Sordellii Toxic Shock (TSS) Syndrome Following Medical Abortion

Dr. Marc Fischer, of the CDC National Center for Zoonotic, Vector-Borne and Enteric Diseases, covered the following areas in his presentation. *C. sordellii* is a gram-positive anaerobic *Bacillus* that typically resides in soil and colonizes the GI and genital tracts of healthy humans. *C. sordellii* is not commonly found in surveys of stool and vaginal flora, but the organism was isolated from musculoskeletal tissues of 3% of cadaver donors in a recent study. Lethal and hemorrhagic toxins determine the virulence and clinical manifestations of *C. sordellii*. However, lethal toxin is variably expressed by different *C. sordellii* strains and its cytopathic effects are altered by environmental conditions.

Case reports have described *C. sordellii* as a cause of pneumonia, endocarditis, arthritis, peritonitis, corneal ulcer, bacteremia and wound infections, including necrotizing fasciitis, myonecrosis, tissue allograft infections, neonatal omphalitis, postpartum endometritis and episiotomy infection. Fulminant TSS among previously healthy persons has been reported in only a small proportion of *C. sordellii* infections. *C. sordellii* TSS is an acute and rapidly progressive disease that is characterized by a lack of or minimal fever; refractory tachycardia and hypotension with no response to intravenous fluids; local edema at the infected site with subsequent pleural and peritoneal fusions; a marked leukemoid reaction; and an elevated hematocrit due to hemoconcentration. *C. sordellii* TSS is fulminant and most often fatal.

From 1976-1993, six cases of *C. sordellii* toxic neonatal omphalitis were reported in the literature among infants 2-11 days of age. These cases were characterized by severe abdominal wall swelling, periumbilical erythema and discharge, and markedly elevated WBC counts. *C. sordellii* was isolated from the umbilicus, peritoneal fluid and blood. Five of these six patients died. From 1992-2000, four clusters of *C. sordellii* wound infections were reported among black tar heroin injecting drug users. Many of the infections contained *C. perfringens* and other soil contaminants. The presence of *C. sordellii* appeared to be associated with a toxic shock-like syndrome and a high case fatality rate.

In 2001, fatal *C. sordellii* sepsis and TSS were reported in a previously healthy male 23 years of age who received a contaminated tissue allograft. The case prompted a broader investigation that identified 13 additional cases of *C. septicum* or *C. bifermentans* infections associated with tissue allografts. None of the 13 cases were fatal. From 1977-2001, ten cases were reported in the literature of female genital tract infection and fatal TSS associated with *C. sordellii* among previously healthy women 23-40 years of age. Of the ten cases, all were fatal, eight occurred after delivery of live-born new infants, one occurred after a medical abortion, and one was not associated with pregnancy. Leukemoid reaction and hemoconcentration were the hallmark findings of these cases.

In 2000, FDA approved mifepristone plus misoprostol for medical termination of pregnancy up to seven weeks gestation. Mifepristone is a synthetic steroid with anti-progesterone and anti-glucocorticoid effects, while misoprostol is a prostaglandin analog that causes uterine contractions. The FDA-approved regimen is 600 mg of oral mifepristone followed within two days by 400 µg of oral misoprostol. From September 2003-June 2005, FDA received reports of four deaths among women who had recently undergone medically-induced abortions (MIAs) with mifepristone and misoprostol. The four patients received a common off-label regimen of 200 mg of oral mifepristone followed by 800 µg of vaginal misoprostol.

The initial investigation found that each case was consistent with fulminant toxic shock-like syndrome. In March 2004, FDA contacted the CDC Unexplained Deaths Project to assist in identifying a specific infectious etiology of the cases. The four women were white, black and Asian; 18-34 years of age and previously healthy; and residents of and obtained MIAs in California. The median time from receipt of mifepristone to onset of initial symptoms was five days. The time from hospitalization to death was <24 hours.

Of the four cases, one had a documented fever; none had a rash; all reported tachycardia, hypotension, vomiting or diarrhea, and severe abdominal pain; three had a significant leukemoid reaction; two had significant hemoconcentration; two had moderate thrombocytopenia; one had renal insufficiency; and none had elevated hepatic enzymes or bilirubin. Blood cultures performed on three patients prior to antibiotics were negative for bacteria. One vaginal swab obtained pre-mortem grew *Gardnerella* species. An endometrial tissue collected postmortem from one patient grew *Escherichia coli* and an anaerobic gram-positive *Bacillus*.

CDC's analysis of fixed tissues from autopsies showed pleural or peritoneal effusions and diffuse pulmonary edema in three of the four cases. None of the patients retained fetal or placental tissue. Hematoxylin and eosin stains of uterine tissue showed

extensive acute inflammation and necrosis of the endometrium and myometrium in all four patients. Three patients had areas of edema and hemorrhage within the uterus. Two patients had multiple abscess formation.

Immunohistochemistry results on uterine tissue were positive in all four patients for *Clostridium* species; positive in one patient for *Staphylococcus aureus*; and negative in all four patients for both group A streptococcus and *Neisseria* species. Mixed bacteria were seen on gram stains of uterine tissue for all four patients, but abundant gram-positive bacilli were the predominant findings in each case. Histopathologic findings for all tissues other than the uterus were unremarkable. Extensive bacilli and granular antigens were seen in the areas of inflammation in the endometrium and myometrium.

Polymerase chain reaction (PCR) results were positive in all four patients for the broad-range 16S rRNA, *C. sordellii* 16S rRNA, *C. sordellii* cytotoxin L, and *C. sordellii* phospholipase C genes. The PCR results on uterine tissue were negative for the *C. perfringens* alpha toxin gene in all four patients. Based on the findings of the investigation, CDC concluded that the four deaths were attributed to *C. sordellii* endometritis and TSS. The clinical and pathologic findings were similar to ten other cases of *C. sordellii* genital tract infections previously reported in the literature. The cases demonstrated that serious infection can occur after MIAs.

Several issues are still unresolved regarding the possible association between the use of mifepristone and misoprostol and the risk of *C. sordellii* TSS, including changes in vaginal flora or environment, incomplete abortion with necrotic decidual tissue, immunosuppressive effects of mifepristone, increased pathogen virulence, altered host susceptibility, and the interaction of multiple factors. Product contamination was eliminated as a cause because no epidemiologic links were identified among the patients. Medications in the four cases were obtained from different clinics, healthcare providers and manufacturing lots. FDA tested both mifepristone and misoprostol from manufacturing lots received by the patients and found no contamination with *C. sordellii*.

A reporting or case detection bias was also considered as potential cause. Local and regional attention in California may have increased awareness among healthcare providers and the public and stimulated reports of additional cases that may have not been detected in other states. No centralized reporting system has been developed for pregnancy-associated infections or deaths. Laboratory confirmation of *C. sordellii* in the four cases resulted from extraordinary efforts by a national reference laboratory. FDA's MedWatch system initially detected the four cases and is designed to identify adverse events associated with approved medications.

In an effort to address these issues, CDC and its partners initiated several supplemental surveillance activities to identify additional cases of severe infection or TSS associated with pregnancy, childbirth or abortion. Articles were published in the *Morbidity and Mortality Weekly Report* (*MMWR*) and *New England Journal of Medicine* requesting that healthcare providers report any TSS cases associated with pregnancy or abortion to local health departments. Specific queries were sent to members of the Infectious Disease Society of Obstetrics and Gynecology (IDSOG), the National Association of Medical Examiners, and CDC's Unexplained Deaths Project sites. The California Department of Health Services initiated a retrospective review of death certificates to identify additional cases.

CDC hopes the workshop will be used as an opportunity to develop a research agenda and answer three key questions. One, are women who use mifepristone or misoprostol at increased risk of *C. sordellii* infection or TSS compared to other women following surgical abortion, spontaneous abortion or childbirth? Two, what is the mechanism of the increased risk? Three, is the risk limited to *C. sordellii* compared to other *Clostridium* or anaerobic bacteria? Four, what approaches can be taken to further reduce the risk and improve treatment of *C. sordellii* infections?

An OB/GYN View of Early Medical Termination and *C. Sordellii* Infection

Dr. James McGregor is a Visiting Professor of Obstetrics and Gynecology at the Keck School of Medicine. He covered the following areas in his presentation. Seven deaths from 560,000 early medical terminations that have been reported since 2000 demonstrate that this risk is higher than surgical termination. The traditional microbiologic paradigm may now be inadequate because the role of innate and acquired immunity and medications should also be considered. Three cases of *Clostridia* were unclassified in a 2005 published study that used basic nucleic acid techniques to identify the microbiology in the vagina among 78 patients.

In animals, *C. sordellii* is associated with malignant edema, bloat, hemorrhage and sudden death in sheep. In humans, *C. sordellii* causes pregnancy-associated infections, wound infections, edema and lethal toxic shock-like syndrome. *TcsL* is a large clostridial toxin that affects glycosylates; the Ras, Rac and Ral genes; and signaling within endothelial and myocardial cells. Previous research showed that increased acid pH disassociated and increased the potency of toxins. Intoxification increased five-fold and the intoxicating dose was lowered by one log at the optimal pH range of effect of 4.0-5.0. These findings suggest that local conditions in the uterus are important factors to consider in identifying *Clostridia* production.

Mifepristone was developed in 1981 and has generated >700 publications. The drug uses progesterone-dependent processes and is applied in the termination of early pregnancy, inhibition of follicular development, cervical ripening, glucocorticoid-dependent processes, breast cancer treatment, labor induction and endometriosis. However, mifepristone's unusually long half-life of 30 hours poses problems in titrating doses within a therapeutic range. Previous research on the pharmacokinetics of mifepristone among five females showed that the drug actively binds to the progesterone receptor in the endothelium and the affinity for glucocorticoid receptors. The affinity of mifepristone was found to be four-fold of dexamethasone.

Another study demonstrated that the effects of mifepristone persisted for one week and was excreted by methylation and hydroxylation. Metabolites are active in terms of both the progesterone and glucocorticoid receptors. A seminal study that was published in 1986 recommended close medical supervision in using mifepristone due to the potential for prolonged and abnormal bleeding. A study published in 2002 found mifepristone and misoprostol to be superior. The study further concluded that mifepristone alone may be acceptable when misoprostol is unavailable.

A retrospective study that was published in 2004 determined that medical early pregnancy termination can be accomplished without mifepristone. Misoprostol was found to be a satisfactory agent for medical abortion at a rate of 88%-96% in this setting. The drug was also administered to 34 patients during the study. Numerous studies on the innate immune response have demonstrated increased lethality in animal models with mifepristone. The drug enhanced *C. difficile* toxin A intestinal secretion and inflammation in a well-controlled clinical trial.

A letter was published in the *Annals of Pharmacotherapy* in 2005 to point out that vaginal application of misoprostol is prohibited in France where misoprostol can only be prescribed in oral form. However, other data from the Netherlands are well reported and serve as an excellent source of solid epidemiological information. A suggestion has been made for physicians in North America to more freely administer antibiotics at the initiation of a medical termination and give steroids if a patient becomes ill. However, other primary prevention strategies should be considered as well.

Consideration should be given to reducing or entirely eliminating mifepristone. The drug should only be administered orally as approved by FDA. Short- or long-course antimicrobial prophylaxis is not likely to be effective. Probiotics will most likely have minimal efficacy from a physiological perspective. Informed consent forms for misoprostol should be changed to more clearly acknowledge potential risks for serious

infection or complications. Treatment and recognition of *C. sordellii*-associated TSS should be improved with a case definition and clearly described laboratory factors.

Patients with *C. sordellii*-associated TSS should be rapidly admitted to a healthcare facility, given consultations with infectious disease physicians and intensivists, and promptly provided with multiple organ support. A hysterectomy or dilation and curettage should be immediately considered for patients with a clear diagnosis of *C. sordellii*-associated TSS. Clindamycin, imipenen, IV/Ig protein C aspects and steroid support should be considered as well.

Modern technologies should be applied to study the sub-cellular, cellular organ and organismal effects of mifepristone and misoprostol and increase knowledge about the active epidemiology of *C. sordellii* in pregnant women and other populations. Existing models of mifepristone should be analyzed to conduct additional research on the pathophysiology of infection and inflammation. Studies should also be conducted on mifepristone and misoprostol metabolism. Active surveillance should be performed on the adverse effects of these drugs.

Clinical Settings, Diagnostic Clues and Pathogenic Mechanisms of *C. Sordellii*

Dr. Dennis Stevens, of the Boise, Idaho VA Medical Center and the University of Washington School of Medicine, covered the following areas in his presentation. *C. sordellii* is an anaerobic organism that was initially isolated in 1937. The gram-positive rod forms spores and was traditionally believed to be a virulent form of *C. bifermentans*.

In one case of *C. sordellii* infection, a male 4 years of age broke his arm in a fall. Pain and marked swelling were noted with no fever, normal blood pressure and a rapid pulse. Cefazolin was administered intravenously and a volar fasciotomy was performed. Necrotic muscle and fascia were later revealed and tissue samples grew a culture of *C. sordellii*. The WBC count rose to 41,000 with increased hypotension, tachycardia and metabolic acidosis. The patient died within three days after the fall.

In another case of *C. sordellii* infection, a female 21 years of age had a vaginal laceration during natural childbirth. Perineal pain increased, the temperature was normal, blood pressure was low, the pulse was 132 and the WBC count was 67,000. Gentamicin and clindamycin were administered intravenously, a fasciotomy of the vulva was performed, and vancomycin was added. The WBC count rose to 123,000 post-operatively. Hypotension, metabolic acidosis and super-ventricular tachycardia developed with a pulse of 170. Copious amounts of intravenous fluids were

administered, but the patient went into cardiac arrest and died. The tissue cultures grew *C. sordellii*.

A database search of reported *C. sordellii* infections yielded 28 reports due to postpartum or obstetrical/gynecological (OB/GYN) infections, MIAs, spontaneous abortions, injection drug use, trauma, surgical procedures or "other" causes. Of 41 patients 17 days to 95 years of age, 49% were males and 51% were females. The data review demonstrated that *C. sordellii* is a deadly disease for females due to postpartum and OB/GYN infections. The most significant factors in the cases were non-elevated temperatures, high WBC counts, hypotension, leukemoid reactions, hemoconcentration with a rising hematocrit, and non-*C. sordellii* organisms.

Autopsy findings of the cases showed local necrosis and acute inflammation; marked tissue or visceral edema; pericardial, pleural or peritoneal effusion; thrombosis of localized blood vessels; and neutrophil degeneration at margins of necrotic tissue. Potent exotoxins clearly play a role in the pathogenesis of *C. sordellii*. Investigations are underway focusing on mechanisms of the capillary leak syndrome and leukemoid reaction. These processes significantly contribute to both *C. sordellii* and *C. difficile*.

In a cell proliferation assay, HL-60 cells were utilized; *C. sordellii* toxins were collected from stationary phase cultures; ammonium sulfate precipitation was developed with isoelectric focusing; and fractions were created with different isoelectric points from 3-10. The cells were exposed to various fractions and were identified by flow cytometry. In an endothelial cell permeability assay, primary human umbilical vein endothelial cells were cultured on a membrane-lined insert to confluency. Permeability was measured by electrical resistance across the membrane. Toxins were added and resistance was measured over a 12 hour-period. A striking difference was seen in the degree of permeability of the endothelial cells and the rapidity of onset.

The investigation is also focusing on the innate immune recognition and response to *C. sordellii*. HEK-293 cells were transfected with genes for toll receptors 1, 2, 4 and 6 individually and collectively. The cells were also transfected with MD2, CD14 and ELAM-1-dependent luciferase reporter systems. Cytokines were measured from peripheral blood mononuclear cells stimulated in parallel. The data showed that toll receptor 4 was used exclusively by LPS and not by clostridia, the exception being *C. septicum.* Toll receptor 2 and a combination of toll receptors 2 and 6 were found to be optimal for the remainder of the *Clostridia* species. Receptors on immune cells that recognize *C. sordellii* in the absence of any acquired immunity were identified.

C. sordellii was found to be equally as potent as lipopolysaccharide (LPS) in terms of cytokine induction. The *C. sordellii* toxin crude preparation was a very potent inducer of IL-1β, but lethal toxin was not. Granulocyte macrophage-colony stimulating factor (GM-CSF) was induced by *C. sordellii* toxins, but was not produced by the lethal toxin. Interleukin-10 was produced to a greater extent by lethal toxin compared to the cruder preparation. The important pathogenic mechanism in *C. sordellii* is the diffuse capillary leak syndrome that appears to be related to direct toxin effects on endothelial cells. The leukemoid reaction appears to play a role in the synergistic interaction between GM-CSF and the ability of a *C. sordellii* toxin to stimulate proliferation of bone marrow progenitor cells.

The Pathophysiology of Mifepristone-Induced Septic Shock Due to *C. Sordellii*

Dr. Ralph Miech is an *Emeritus* Associate Professor in the Department of Molecular Pharmacology, Physiology and Biotechnology at Brown Medical School. He covered the following areas in his presentation. Mifepristone may have contributed to the deaths of four healthy women in California from septic shock due to *C. sordellii* infection following medical abortions. In all four cases, the women were <2 months pregnant, given a single dose of 200 mg of mifepristone orally, and self-administered 800 µg of misoprostol vaginally 24-48 hours later. All four women died within a five- to seven-day time period with clinical signs of shock, absence of fever, leukocytosis and hemoconcentration.

Most drugs are eliminated from the body in a few hours, but pharmacokinetic studies have shown that the long half-life of mifepristone is generally on the order of 20-30 hours. As a result, four to five days would be needed to remove 95% of the drug from the body. Other data have demonstrated that some humans have an unusually long half-life and would need 18 days to remove 95% of mifepristone. Mifepristone is principally removed from the body by metabolism.

Of six different metabolites of mifepristone that have been identified, some have retained biological activity as progesterone antagonists. However, further research is needed on the effects of these six metabolites on the innate immune system. *In vitro* studies with liver microsome enzymes have shown that the cytochrome P450-3A4 enzyme is primarily responsible for metabolizing mifepristone. Evidence also suggests that the enzyme can be inactivated during the metabolism of mifepristone and accounts for the relatively long biological half-life in humans.

Mifepristone binds with high affinity to both progesterone and anti-glucocorticoid receptors and blocks cortisol receptors in peripheral tissues and the central nervous system. Blockade of negative feedback receptors in the hypothalamus results in increased serum levels of ACTH and cortisol. Experimental animal protocols have demonstrated that mifepristone produces a temporary drug-induced adrenalectomy. Mifepristone was originally developed as an anti-glucocorticoid for the treatment of Cushing's disease. The drug was later discovered to possess anti-progesterone activity and act as an abortive agent.

Mifepristone's anti-progesterone activity results in pharmacological actions on the pregnant uterus, including cervical ripening, ischemia of the decidua, necrosis of the products of conception, and sensitization of the myometrium to contraction by prostaglandins. *C. sordellii* and other *Clostridium* species have been found in the normal vaginal flora in 8%-18% of women. Macrophages, monocytes, neutrophils and endothelial cells serve as the first line of defense for a host to counter bacterial invasion of the interstitial space of uterine tissue. In the reported cases, mifepristone anti-glucocorticoid action initially impaired the proper functioning of the cells of the innate immune system within the pregnant uterus and ultimately led to a uterine infection with *C. sordellii*.

Lipoteichoic acid and peptidoglycan molecular components are unique to cell walls of anaerobic bacteria. These unique biochemical entities bind to and activate toll-like receptors on tissue macrophages. Toll-like receptors function as principal sensors of infection in mammals when activated and cause an outpouring of pro-inflammatory cytokines at the site of bacterial invasion. Tumor necrosis factor alpha and interleukins-1 and -6 are the principal pro-inflammatory cytokines that are synthesized and secreted by phagocytes.

When inflammation is properly controlled, invading bacteria are destroyed without tissue damage. However, *C. sordellii* can successfully establish an infection with the secretion of lethal toxin. Excessive pro-inflammatory cytokines can gain access to the systemic circulation if local reaction of pro-inflammatory cytokines is not controlled. Both of these events contribute to the etiology of septic shock and multiple organ dysfunction. The proper timing and amount of cortisol are crucial to maintaining control of the inflammatory response and preventing tissue damage.

Cortisol binds to intracellular glucocorticoid receptors and phagocytes to cause increased transcription of DNA. This process results in the synthesis and secretion of the interleukin-10 anti-inflammatory cytokine. Interleukin-10 suppresses the generation

of excessive pro-inflammatory cytokines, tumor necrosis factor alpha, and interleukins-1 and -6 by cells of the innate immune system.

Animal experiments support the hypothesis that mifepristone can facilitate infection and lead to lethal septic shock. A study showed that a single dose of the drug administered to mifepristone-treated mice dramatically increased the mortality rate of polymicrobial septic shock nearly three-fold. Mifepristone may impair the innate immune system to delay proper removal of *C. sordellii* from the decidua and allow bacteria to secrete lethal toxin into the uterine interstitial fluid.

Phagocytes, endothelial cells and uterine cells allow entry of lethal toxin and prevent proper participation in defensive inflammatory responses of the innate immune system. Lethal toxin functions as an intracellular enzyme that catalyzes the glucosylization of G-proteins when internalized by uterine phagocytes and internal endothelial cells. G-proteins are molecular switches that activate or inhibit a multitude of vital biochemical cascades and genetic transcription functions for proper functioning of cells. Glucosylization causes G-proteins in uterine phagocytes to become useless in destroying bacteria.

Overall, mifepristone's anti-progesterone effects prepare the aborting uterus as an ideal bacterial culture for *C. sordellii* by causing ischemic decidua that leads to necrotic products of conception. Mifepristone's anti-glucopharmacologic actions disrupt the hypothalamic pituitary adrenal axis and interfere with the functioning of peripheral glucocorticoid receptors at a crucial time. This process results in a lack of control of the pro-inflammatory cytokine response and allows for the establishment of a nidus of infection with *C. sordellii* and localized secretion of lethal toxin.

Phagocytes in the decidua are permanently inactivated by lethal toxin and allow *C. sordellii* to multiply and secrete excess lethal toxin into the systemic circulation. The combination of lethal toxin and excess inflammatory cytokines in the systemic circulation can synergistically collaborate to produce clinical findings of rapid fulminating lethal shock syndrome that were the hallmark of the four cases in California.

Bacterial Toxin Repression of Nuclear Hormone Receptors: Host-Pathogen Hormone Interactions and Implications for Therapy

Dr. Esther Sternberg, of the NIH National Institute of Mental Health, covered the following areas in her presentation. Hormones have profound effects on host inflammatory and immune responses. Research conducted in 1989 showed that

blocking the hypothalamic pituitary adrenal axis predisposes rats to increased inflammation because glucocorticoids play an extremely important role in regulating the inflammatory and immune response. The hypothalamic pituitary gonadal axis is also significant in regulating inflammation and the immune response. Progesterone and glucocorticoids both have immunosuppressive effects that can shift a response from T_H1 to T_H2.

Estrogen typically stimulates a TH1 or cellular pattern of immune response at low concentrations. At high concentrations, estrogen, progesterone and glucocorticoids cause a shift from a TH1 to a TH2 pattern of immunity that is a humoral or antibody pattern. Estrogen and progesterone vary throughout the menstrual cycle with progesterone peaking during the luteal phase. Several studies have shown that immune function changes are associated with decreased T-lymphocyte chemokine receptors, increased antibody production, and increased infection susceptibility to a variety of infectious agents.

The data also demonstrate that these hormones generally increase throughout pregnancy and peak in the second and third trimesters. Associated immune function changes have been reported during pregnancy with strong immunosuppression, increased suppressor T-cells, decreased cytotoxic T-cell function, increased antibody production, and increased susceptibility to infections such as *Toxoplasma gondii*. Many pregnancy studies focus on the effects of these hormone changes in association with changes in symptoms of autoimmune diseases.

In one study (unpublished), flow cytometric analysis showed that dendritic cells express glucocorticoid and progesterone receptors. This study also demonstrated that progesterone is generally inhibitory on dendritic cells; does not affect immature dendritic cells; primarily suppresses pro-inflammatory TNFα production in mature dendritic cells; has no effect on anti-inflammatory interleukin-10 production; and down-regulates MHC-II and CD80 co-stimulatory molecule expression.

Unpublished studies on the effects of progesterone on TNFα secretion in rat bone marrow-derived dendritic cells showed that LPS induces TNFα production. Increased concentrations of progesterone were found to inhibit TNFα production. Mifepristone was shown to prevent progesterone inhibition of TNFα production in vitro.

Research was conducted to determine whether bacterial toxins interact with host hormone responses. Studies on *Bacillus anthracis* lethal toxin demonstrated in a transient transfection system, that lethal toxin repressed transactivation of the glucocorticoid, progesterone and other nuclear hormone receptors. The repression

occurred at very small concentrations of lethal toxin and was both receptor- and promoter-specific.

Further studies (unpublished) also found that *C. difficile* and *C. sordellii* toxins repressed the glucocorticoid receptor. Other unpublished research showed that *C. sordellii* lethal toxin (TcsL) partially prevents dexamethasone suppression of splenocyte TNFα production. The effect of blocking glucocorticoid transactivation that was observed with TcsL also translated to an effect in the physiological system in preventing dexamethasone suppression of TNFα production.

Sub-pharmacological concentrations of mifepristone plus the *C. sordellii* toxin completely reversed dexamethasone suppression of TNFα production *in vitro*. However, further research must be conducted to determine whether *in vitro* findings translate to an *in vivo* setting. A study that was published in 1989 demonstrated that mifepristone resulted in 100% mortality in a strain-specific manner when given in combination with streptococcal cell walls to otherwise inflammatory resistant Fischer rats. Mifepristone alone had no effect in this study.

Other methods to block the hypothalamic pituitary adrenal axis have been applied in more recent studies in animals exposed to other pathogens or pathogen products. These include adrenalectomy and hypophysectomy and exposure to pro-inflammatory antigens or pathogens, such as salmonella, murine cytomegalovirus (MCMV) virus or shiga toxin. This research has also shown greatly enhanced mortality when the hypothalamic pituitary adrenal axis is blocked and the animal is exposed to pro-inflammatory triggers or pathogens. Glucocorticoids were found to reverse or prevent this effect in most cases.

Recent studies were conducted on the effects of anthrax lethal toxin in mice. Adrenalectomy of otherwise anthrax-resistant mice greatly enhanced mortality in the animals. Dexamethasone did not rescue the adrenalectomized mice from anthrax lethal toxin mortality and instead enhanced mortality. We postulate that the lack of effect of glucocorticoids is related to the mechanism of action of the toxins, which attack the glucocorticoid receptor and fundamentally inactivate the receptor. The results emphasize the critical need to test these types of questions *in vivo* before treatment is applied to humans. Moreover, studies should be conducted focusing on specific toxins and hormones of concern before treatment with glucocorticoids is recommended.

The anthrax lethal toxin research showed that mifepristone had variable effects on *Bacillus anthracis* lethal toxin mortality in mice. These findings suggest that adrenal factors other than glucocorticoids play a role in mortality related to this toxin. Overall,

glucocorticoids and progesterone generally suppress inflammatory responses. *Clostridia* bacterial toxins partially repress glucocorticoid receptor transactivation. *Clostridia* bacterial toxins partially reverse dexamethasone suppression of TNFα production. Sub-pharmacological concentrations of mifepristone plus TcsL completely reverse the effects of dexamethasone suppression of TNFα production *in vitro*.

The priority in future research will be to determine if *in vitro* findings can translate to *in vivo* situations of shock. Emphasis should be placed on the time course after exposure to bacterial products; an individual's dose-response and exposure to hormones and drugs; prostaglandins or other drug interactions; and pregnancy and menstrual cycle hormone levels, receptor polymorphisms or mutations that may make some persons more susceptible to inhibitory effects of other factors blocking these receptors.

The published literature is increasingly demonstrating that glucocorticoid receptor polymorphisms or mutations in associated proteins necessary for the glucocorticoid receptor to function are prevalent in numerous autoimmune and inflammatory diseases. These polymorphisms and mutations may also play a role in enhanced inflammatory responses in certain individuals. Host factors, bacterial product interactions with the host, and immune and hormonal responses that interact with these factors must all be considered in future research to identify risk factors to prevent these occurrences.

Panel 1-Session 2 Discussion

The Panel 1-Session 2 presenters provided additional details about the clinical syndromes, pathophysiology and host factors of *C. sordellii* in response to questions and comments from the participants.

- Broad spectrum or focused antimicrobial agents should not be given for *C. sordellii* because this occurrence is still rare. Administering antimicrobial agents with activity against *C. sordellii* while initiating the medical termination should not be considered because multiple changes in the microecology of the patient would be provoked. For example, 10% of patients would immediately develop yeast infections and have changes in the GI flora.
- A hypothesis to link *C. difficile* and *C. sordellii* in the pregnant patient should be tested in animal models. For example, a pregnant woman would be more at risk for infection if progesterone is elevated and is found to be an equally important anti-inflammatory factor in *in vivo* studies

compared to *in vitro* studies. The patient may develop shock if certain bacteria producing these toxins generate no greater growth and toxins inhibit inflammatory responses that are intended to protect the host from bacteria.

- *C. sordellii* spores, microorganisms and other substances from the vagina are transported inside the uterus when the uterus contracts. These factors and prolonged bleeding during medical treatment are extremely important to consider during intravaginal administration of misoprostol. Denmark, Sweden and the Netherlands have developed nationalized medical systems and better tracking processes than the United States. Efforts should be made to review and collect epidemiologic data from these countries.
- *C. sordellii* cases have been reported in the literature, but the epidemiology has not been identified at this point because these serious infections are still rare. Speculations have been raised that *C. sordellii* infections may be attributed to an individual's predisposition, the actual agent used or the polymorphism for the P450 enzyme.

PANEL 2: SURVEILLANCE FOR DISEASE AND SOURCES OF INFECTION

The Panel 2 speakers made presentations to identify current and future surveillance needs and barriers to disease and sources of infection. The two Panel 2 presentations are outlined below.

Federal and International Initiatives

Dr. Clifford McDonald, of the CDC Division of Healthcare Quality Promotion, covered the following areas in his presentation. Most CDAD is acquired in healthcare facilities, but these infections are not nationally reportable and currently require alternative sources of national data to determine disease rates. CDC's National Hospital Discharge Survey showed that CDAD discharge diagnosis among U.S. hospital patients doubled between 2000-2003 and increased an additional 25% in 2004.

Other countries are also making efforts to survey CDAD in hospitals. In August 2004, Quebec instituted mandatory reporting of CDAD for acute care hospital cases, including cases with symptom onset $\leq$1 month post-discharge, major complications and death. Canada performed two national sentinel surveys in 1997 and 2005 to determine CDAD

rates and also collected *C. difficile* isolates from facilities in 2005. In January 2004, England instituted mandatory reporting of CDAD among all patients ≥65 years of age for all healthcare facilities within individual national health system trusts.

CDC has taken several actions to prioritize and promote surveillance of HA-CDAD. Surveillance recommendations were developed and are currently being reviewed by external partners. The guidance will formally state CDC's communications on its web site and public presentations. Definitions and methods that should be used to report infections will be clearly outlined. The recommendations will be targeted to healthcare facilities and networks, state health departments and public reporting initiatives. All healthcare facilities are advised to conduct some type of surveillance.

CDC is using its EpiCenter hospital grantees to analyze surveillance methods and is also developing a CDAD surveillance component for inclusion in the National Healthcare Safety Network. A 2005 *MMWR* article featured reports of CA-CDAD among 23 generally healthy persons who had no recent exposure to healthcare facilities. Several of these individuals also had no recent antimicrobial use. An increase in CA-CDAD among persons who sought care at the VA Hospital in Atlanta, Georgia was also reported to CDC. PPIs appeared to increase the risk for CDAD in these patients.

A 2005 *MMWR* article described ten cases of pregnancy-associated CDAD from four states that resulted in severe disease and one death. CDC and the Emerging Infections Network of the Infectious Disease of Society of America are collaborating to actively investigate additional cases of pregnancy-associated-CDAD. Of 405 infectious disease clinicians who responded to a survey, 4% reported having seen cases and 6% were aware of cases in their respective communities. Of the 48 cases of pregnancy-associated CDAD reported through the survey, 29% occurred prior to delivery; 20% developed recurrent disease; three developed toxic megacolon; one resulted in fetal loss; and one resulted in maternal death.

Since 2000, CDAD outbreaks have been reported in food-producing animals, particularly neonatal pigs, beef and dairy calves. The strains infecting animals are genetically different from the most common human strains. The same PCR ribotype is causing recent outbreaks in both pigs and calves, but is not the traditional ribotype among human isolates. However, the epidemic animal strains share certain characteristics with the recently described human epidemic strain and may indicate increased virulence. Both the human and food animal epidemic strains carry the binary toxin and toxins A and B and have a deletion in the putative *tcdC* toxin regulatory gene.

The human epidemic strain has an 18-base pair *tcdC* deletion, while the food animal strain has a 39-base pair deletion.

CDC is aware of human CDAD cases that are caused by strains similar to animal epidemic strains. Pulsed-field gel electrophoresis genetic typing shows that animal and human isolates are at least 80% related. CDC's ongoing investigation indicates that five of seven human CDAD cases caused by animal epidemic strains fit a clinical picture typical. The cases are primarily occurring in healthcare facilities among older patients with significant co-morbid diseases. One death attributed to CDAD occurred in a younger patient without co-morbidities and may have been community-associated.

Disease occurring in food animal production facilities is primarily due to animal-to-animal transmission, but the existence of environmental sources or reservoirs for responsible stains is not known at this time. The transmission dynamics between food animals and humans are also unknown. CDC is partnering with FoodNet under the Emerging Infections Program (EIP) to investigate CA-CDAD and attempt to fill data gaps. Pilot studies were initiated to obtain isolates from community cases and perform cultures on retail meat samples.

For *C. sordellii*, CDC is actively investigating four additional (and previously unreported) cases of pregnancy-associated toxic shock-like syndrome. Of these four cases in which the pregnancy outcome was either medical or spontaneous abortion, all were <35 years of age, occurred since 2000, and three died. Of the five cases of toxic shock-like syndrome following medical abortion previously reported in the United States and Canada, all received mifepristone and intravaginal misoprostol 6-10 weeks gestation and developed a *C. sordellii* intrauterine infection. Of three additional medical abortion-related cases that CDC is still investigating, two occurred in the Western United States, at least one was known to have not taken mifepristone (but used laminaria and misoprostol instead), two were given intravaginal misoprostol, and two had *Clostridium perfringens* infections. CDC has been unable to confirm that a medical abortion was indeed performed for one of the patients.

Of the two previously reported cases of toxic shock-like syndrome following miscarriage, both occurred in the second trimester, both cases had a *C. sordellii* infection, and one had a *C. perfringens* infection. CDC's ongoing investigation of one additional case showed that *C. sordellii* recovered from blood did not contain genes encoding lethal toxin. Various passive case finding methods were used to notify CDC about the pregnancy-associated toxic shock-like syndrome cases, including FDA adverse events monitoring, reports from state health departments and academic partners, and the CDC Division of Reproductive Health Pregnancy Mortality Surveillance System.

The surveillance system prevents deaths by monitoring trends and identifying risk factors associated with deaths. However, clinical and pathology samples cannot be requested and identifiable information cannot be published due to privacy rules. CDC is collaborating with public health partners in California to identify additional cases of pregnancy-associated toxic shock-like syndrome. Death certificates of women 15- 44 years of age are being reviewed to identify anaerobic septicemia, TSS, inflammatory disease of female pelvic organs, or other indications of pregnancy-associated deaths. The death certificate review has identified 321 potential cases from 2000-2003.

CDC has tested a bank of isolates submitted over the past 30-40 years and confirmed that only a minority of clinical *C. sordellii* possess lethal toxin. These data show no increase in the number or proportion of isolates that are toxin-positive. Isolates that CDC genetically typed showed no evidence of epidemic *C. sordellii* strains. Only a few of these isolates appeared to be highly related to one another and no clustering was seen of toxin-positive versus toxin-negative strains. CDC acknowledges the critical need for further research on the epidemiologic sources and transmission dynamics of *C. sordellii.*

State Initiatives and Performance Characteristics of Optimal Surveillance Systems

Dr. Jeffrey Engel is the North Carolina State Epidemiologist in the North Carolina Division of Public Health. He covered the following areas in his presentation. The United States currently utilizes the passive Nationally Notifiable Disease System (NNDS) to report communicable diseases. Most states have laws that mandate physicians and laboratory directors to report communicable diseases. States also develop rules with a list of reportable diseases and specific mechanisms to submit reports.

North Carolina and several other states allow county health departments to be autonomous in reporting diseases, while other states institute regional reporting by health districts. Diseases are reported by event codes and extracted from confidential medical records. Public health departments are authorized to obtain all confidential records on any mandatory reportable disease in the state. However, disease reports can be discoverable in response to a Freedom of Information Act request or an individual's signed consent and release form. Public health departments also have authority to investigate non-reportable diseases and emerging infections, such as CDAD or *C. sordellii*.

One of three approaches can be taken to place emerging infections or diseases on the NNDS. The federal government can define an "emergency," such as severe acute respiratory syndrome or monkeypox. Other public health threats can be identified, such as pediatric influenza deaths or novel influenza virus. CDC and the Council of State and Territorial Epidemiologists can jointly develop consensus statements for case definitions. North Carolina and many other states require outbreaks to be reported to local health departments. However, hospitals in North Carolina are not mandated to report other diseases because information cannot be protected. As a result, HA infections are not reported to NNDS.

Two states are now taking actions to report CDAD. Connecticut initiated this effort to determine whether toxic strains were emerging in the community. A committee represented by hospitals, laboratories and community members approved the addition of CDAD to the state list of reportable diseases as of January 1, 2006. A descriptive epidemiologic program was piloted to analyze and evaluate trends of community-onset (CO) CDAD. Connecticut's case definition is the onset of illness while residing in the community and no contact with a healthcare or long-term care facility (LTCF) in the previous three months.

CDAD surveillance for the state of Connecticut is performed by infection control practitioners in 31 acute care hospitals and includes an intensive questionnaire, chart review and follow-up at a physician's office. Of the 86 cases investigated as of May 1, 2006, 39 were ruled out, 17 had actual CO-CDAD, and 30 are pending review. Connecticut is collaborating the CDC FoodNet program for the laboratory component of the CDAD surveillance system. CDC now receives cultures from 11 sites across the country and is seeking ten isolates of *C. difficile* organisms from Connecticut. Connecticut's most significant challenges in the CDAD surveillance system are resources and the storage of stool samples in laboratories while cases are being investigated.

Ohio initiated its CDAD surveillance system in response to concerns expressed by citizens and the media regarding CDAD outbreaks in healthcare facilities and a directive by the governor. Mandatory hospital and LTCF surveillance was established on January 1, 2006. At this time, ~200 acute care hospitals and 1,000 nursing homes report numerator data by week. Initial case reports of CDAD are also posted on the Ohio Department of Health web site. Ohio's previous public reports of HA-CDAD required >48 hours after admission, but the new version captures denominator data by bed or patient day.

By June 1, 2006, Ohio's public reports will reflect actual rates by patient day in acute care hospitals and rates by bed or occupied bed day in LTCFs. Risk adjustments will not be made for co-morbidities, age or other factors. Ohio has already benefited from the CDAD surveillance system with the establishment of a secure, web-based and statewide reporting tool. Moreover, the system provides an opportunity to educate healthcare facilities about appropriate antibiotic usage and infection control.

North Carolina requested an EpiAid from CDC in 2005 for assistance in investigating a marked increase of suspected CA-CDAD among veterans. The retrospective study covered the period of January 1-December 31, 2005 with a cohort of four VA Medical Centers, one tertiary care center and one regional hospital in the state. Of the 625 CDAD cases identified at this time, 48% were CO-CDAD and 24% were CA-CDAD. North Carolina's case definition was "no healthcare contact within two months." Of the 58 veterans in the study, the median age was 60½ years, 33% were on PPIs, 16% were on H2 blockers, 19% were on non-steroidal anti-inflammatory drugs, and 50% were on antibiotics. North Carolina is currently investigating all the patients in the study with no antimicrobial exposure.

Several characteristics are necessary for an ideal public health surveillance system. Stakeholders should be engaged in the evaluation. The surveillance system to be evaluated should be described. The evaluation design should be focused. Credible evidence regarding performance of the surveillance system should be gathered. Conclusions should be justified and stated and recommendations should be made. Evaluation findings and lessons learned should be used and shared. To optimize performance of a surveillance system, emphasis should be placed on simplicity, flexibility, data quality, acceptability, sensitivity, predictive value positive, representativeness, stability and timeliness.

Resources must be available for states to conduct surveillance. For example, Connecticut and ten other states are EIP grantees and receive federal funding to participate in the Active Bacterial Core Surveillance and FoodNet Programs and address emerging problems in the United States. The EIP sites will play a critical role in advancing state-level surveillance for *C. difficile* and other new infectious diseases in the future. EpiAids from CDC are free of charge and will continue to serve as a surveillance resource to states.

Panel 2 Discussion

The Panel 2 presenters provided additional details about the current and future surveillance needs and barriers to disease and sources of infection in response to questions and comments from the participants.

- Priorities for *C. difficile* disease surveillance at the federal level include continued emphasis on HA-CDAD; increased understanding of emerging forms of disease; the development of standardized surveillance guidelines and parameters; and stronger knowledge on CA-CDAD in special populations. Case finding will continue to serve as the most important priority at the federal level for *C. sordellii* disease.
- Priorities for *Clostridium* disease surveillance at the state level include new legislation for mandatory public reporting of HA infections; the establishment and monitoring of simple infection control interventions to reduce disease; and standardized methods for hospitals to share data.
- Electronic reporting through laboratory tests is the most promising method to conduct surveillance of *C. difficile* at both federal and state levels. A positive laboratory test on a liquid stool could serve as a surrogate for *C. difficile* disease. However, the period of risk of exposure to disease in a hospital must be clearly defined. For example, Canada conducts post-discharge surveillance of *C. difficile* over a four-week period and the European Union is considering a one-month period.
- CDC has found strains of *C. difficile* that are epidemic in food-producing animals and similar strains have been detected at a low rate in humans. Evidence has not been collected to demonstrate transmission through the food supply or any other means. CDC intends to actively investigate the impact of animal health, environmental factors and shared regulatory genes on human health in terms of *Clostridia*.
- CDC evaluates and investigates all reports of adverse events submitted by other countries on MIAs with mifepristone. CDC is extremely interested in collaborating with and collecting tissues from cases that occur in other countries. CDC has engaged other countries in dialogue about potential methods to strengthen case finding.
- CDC estimates that 400,000-500,000 CA-CDAD and HA-CDAD cases occurred based on 2004 data from the National Hospital Discharge Data Set, states, LTCFs and acute care facilities. Of persons with *C. difficile* infection, 1%-1.5% traditionally die from the disease. However, studies on the new strain in Canada show that 30-day mortality is more on the order of 6.8%.

- CDC's surveillance of pregnancy-associated toxic shock-like syndrome showed that *C. sordellii* had the gene for lethal toxins in cases reported in the literature. CDC also determined through its investigations that *C. difficile* is still an infrequent and uncommon disease in pregnant women.
- CDC has not made a clear determination on the importance of PPIs in CA-CDAD at this time because various epidemiologic studies have reached different conclusions related to the role of age, H2 blockers, antibacterial effects and other factors. CDC acknowledges the need to develop a better model to fill this data gap.
- CDC is unable to confirm reports of an increase in *Staphylococcus* TSS. However, data suggest that some methicillin-resistant and CA strains have staphylococcal enterotoxin genes in a greater proportion than HA and methicillin-sensitive strains. Strains endowed with genes that cause TSS are now present. Fluoroquinolones have been associated with an increase in *C. difficile* and serve as a significant risk factor for methicillin-resistant *Staphylococcus*. These agents may serve as the common denominator in this area.

PANEL 3: IDENTIFYING A RESEARCH AGENDA

The Panel 3 speakers recommended research agenda priorities in the areas of surveillance and epidemiology; basic research; and diagnosis, treatment and prevention of *C. difficile* and *C. sordellii*. Remarks by the three Panel 3 speakers are highlighted below.

Dr. Gerding recommended that the following actions be taken to develop a research agenda for the detection and surveillance of *C. difficile* and *C. sordellii* cases.

- Replicate Quebec's surveillance system to gather solid epidemiologic data on *C. difficile* rates and collect isolates.
- Increase knowledge of and access to *C. difficile* isolates by improving the ability of hospitals to perform cultures to isolate the organism.
- Strengthen current capacity to characterize *C. difficile* isolates by typing systems; analyzing isolates for toxin; focusing on virulence factors of the new epidemic strain; and identifying factors other than fluoroquinolone resistance, binary toxin and increased *in vitro* production of toxins A and B in these strains.
- Apply *C. difficile* isolates in animal models to gather comparative data and advance the field.

- Enhance prevention and control of *C. difficile* in hospitals by widely adapting the excellent environmental disinfection capabilities of bleach and hydrogen peroxide.
- Conduct studies to fill data gaps on the lack of sporucidal activity of alcohol gels and the appropriate time to use hand washing versus alcohol gels.
- Perform research to determine whether *C. difficile* rates in hospitals can be affected or controlled by antimicrobial use. Review successes with clindamycin in the healthcare setting in this effort.
- Develop a rapid and sensitive test for *C. difficile.*

Dr. David Soper is a Professor in the Department of Obstetrics and Gynecology and the Department of Internal Medicine at the Medical University of South Carolina. He recommended that the following actions be taken to develop a research agenda for the detection and surveillance of *C. difficile* and *C. sordellii* cases.

- Strongly urge CDC to continue to conduct active surveillance and investigations in California and other states to answer the following questions? (1) What are the geographical locations and specific populations of the *C. difficile* and *C. sordellii* cases from an epidemiologic perspective? (2) What approaches should be taken to determine whether the *C. difficile* and *C. sordellii* cases are actually a widespread problem? (3) Is the current passive pregnancy mortality system adequate to determine abortion deaths? (4) What proportion of the abortion deaths were due to infection or TSS? (5) What is the role of California in the four fulminant toxic shock-like syndrome cases?
- Develop a new resource for infectious disease clinicians to determine the etiology of unknown pregnancy-related deaths among their patients. For example, IDSOG and the Infectious Disease Society of America could be engaged to inform infectious disease clinicians throughout the country that specimens with an unknown cause of pregnancy-related death can be submitted to a central repository. Experts managing the repository could analyze the specimens to identify microorganisms, determine potential cases of *C. sordellii*-associated TSS, and provide the reporting physician with the findings.
- Conduct studies to determine whether a continuum of infection is present in the diagnosis, treatment and prevention of clostridial diseases. Use these results to identify opportunities for therapeutic intervention and determine biomarkers to alert clinicians to the need for aggressive intervention early in disease.

- Perform research to determine the impact of antitoxins. Identify potential opportunities to manufacture an antitoxin and make the drug universally available through IV/Ig or some other uniform mechanism that is on-the-shelf.
- Conduct studies to confirm whether antimicrobial prophylaxis should be recommended for medical abortions. Note that all patients who undergo surgical abortions are given doxycycline or metronidazole and all *C. difficile* data suggest antimicrobial agents increase the risk of infection.
- Perform basic research to identify key components in toxin production of *C. sordellii*, such as interactions with local conditions or medicines; epidemiologic factors associated with pregnancy-related infections; or pregnancy.
- Develop an animal model to determine whether the efficacy of antitoxins actually prevent the overall syndrome.
- Conduct studies focusing on an individual's genetic polymorphisms, response to the toxin or microorganism, and ability to respond to different infectious insults.

Dr. McDonald recommended that the following actions be taken to develop a research agenda for the detection and surveillance of *C. difficile* and *C. sordellii* cases.

- Prioritize the following areas in the research agenda: HA-CDAD for *C. difficile* surveillance in both LTCFs and acute care hospitals; case finding for *C. sordellii* surveillance; and the risk factors and use of PPIs for *C. difficile*.
- Perform research for public health to better define the epidemiology of *C. difficile* and *C. sordellii* and develop appropriate surveillance definitions. Clearly define the time period a patient is likely to manifest disease acquired in the hospital after being discharged.
- Provide guidance on whether CDC's intention to conduct one-month post-discharge surveillance is appropriate or necessary.
- Identify the incubation period of *C. difficile* by conducting studies in which persons are cultured over time and determinations are made on when a new strain was acquired and disease developed.
- Continue to monitor overall clostridial disease rates in pregnant women and other special populations over time.
- Increase efforts to collect more CA-CDAD isolates.
- Replicate large sentinel surveillance systems developed by other countries to periodically collect isolates throughout the United States and remain prepared for future shifts in *C. difficile* virulence.

- Place more emphasis on sources of infection, particularly the food supply and environments in the hospital, home and community.
- Continue to prioritize prevention and infection control of *C. difficile* with hand hygiene and alcohol gels in healthcare facilities. Optimize other strategies to prevent cross-transmission and reduce environmental contamination.
- Develop means by which the public health sector can somehow "fill the gap" left by clinical laboratories largely abandoning anaerobic bacteriology in the United States.

Panel 3 Discussion

The floor was opened for the other panelists and members of the audience to recommend additional actions that should be taken to develop a research agenda for the detection and surveillance of *C. difficile* and *C. sordellii* cases.

- Provide clear guidance to healthcare providers and drug manufacturers in evaluating treatment options for patients and developing a protective and life-saving antitoxin.
- Assess or redesign the current pregnancy mortality surveillance system as a more active tool in identifying risk factors for significant morbidity and mortality related to pregnant women, live births and pregnancy terminations.
- Conduct *in vivo* studies in animal models and structure the research agenda to broadly address the entire spectrum of complex and multiple factors, including the hormonal balance; inflammatory responses; the timing, role and termination of pregnancy; and mutations or polymorphisms in receptors that may make certain individuals more or less susceptible to disease.
- Ensure that the research agenda clearly distinguishes between "sepsis" and "*C. sordellii*."
- Apply findings from cellular experiments to identify the impact of toxins on endothelial function, cardiac function and death.
- Allocate funding for research on the important role of polymorphisms in drug metabolism and the genetic control of inflammation.
- Develop an animal model with the following objectives: evaluate the ability of drugs to inhibit the innate immune system; increase capacity to identify drugs that may contribute to *C. difficile* re-infection; and assess

mifepristone, misoprostol and other drugs with potential contributions to post-abortion infections.

- Increase knowledge and understanding of the microbiology and basic bacteriology of *C. difficile* and *C. sordellii*. Perform research on sporulation; mechanisms by which the organism germinates and initiates disease; nutrients on which the organism grows; and toxin regulation and genome sequence. Use these findings in killing the organism and neutralizing or suppressing the toxin.
- Conduct extremely cautious epidemiologic studies in appropriate settings among pregnant females to analyze *C. sordellii*, identify other postpartum infections, and develop better interventions for women colonized with *C. difficile* or *C. sordellii*.
- Ensure that active case finding is representative of pregnancy-associated deaths or severe illness regardless of whether an abortion was performed. Follow-up these cases with an intensive epidemiologic review to identify uncommon factors, laboratory diagnoses and factors other than cultures.
- Administer a survey or conduct a study among women in early and late pregnancy to determine potential changes over time. Compare women who first present for an obstetrical visit to those who present for an abortion. Apply findings between the two groups to identify differences in epidemiology, persons colonized with *C. difficile* or *C. sordellii*, and the number of toxigenic organisms.
- Perform research on the role of the media in communicating risk, assisting with case finding, and increasing the transparency of issues from both clinical provider and public health perspectives.
- Encourage CDC and FDA to jointly develop a consultation resource for emergency room physicians, OB/GYNs and primary care providers who have questions about potential *C. difficile* or *C. sordellii* cases. For example, practitioners could call a toll-free telephone number to receive accurate information and obtain support on reporting cases.
- Thoroughly review the published literature in developing the research agenda to strengthen understanding of and explore the possible causal relationship among mifepristone, medical abortions and *Clostridia* infections. Widely publish these findings for physicians to recognize, intervene and treat signs and symptoms of toxic shock-like syndrome early in the progression of disease. Provide clear guidance to clinicians on non-specific symptoms of TSS and appropriate clinical judgments on antibiotic treatment versus conservative or aggressive surgery.
- Perform research to identify effective methods to educate and communicate information about case finding and the prevention of

catastrophic outcomes from large clostridial toxins. Target these messages to emergency room physicians, primary care physicians and other non-infectious disease clinicians who will actually treat *C. difficile* or *C. sordellii* cases.

- Place emphasis on the role of large clostridial toxins in abdominal sepsis to measure toxin levels in peripheral blood.
- Educate women on postpartum infections and the potential consequences of pregnancy or MIAs.
- Determine whether federal agencies can focus on other diseases with a similar pathology or pathogenesis that are higher priorities than *C. difficile* or *C. sordellii* and can be translated into a useful animal model.
- Urge FDA to collaborate with France and other countries to enhance knowledge of specific drug use patterns and strengthen case finding.
- Clearly define, validate and use "severity of disease" in the research agenda to better identify and categorize *C. difficile* sources of disease, particularly patients at high risk of complications or fatal outcomes who need more aggressive management. Widely communicate the severity of disease case definition to identify patients as early as possible in the progression of disease.
- Encourage pathologists to perform microbiologic research on fresh tissues to identify cases of *C. sordellii*.
- Conduct studies and develop better genetic tools on the basic physiology of *C. difficile*, particularly spore formation, virulence factors, spore germination and the genome sequence.
- Partner with the National Association of Public Health Veterinarians and the U.S. Department of Agriculture to determine the role of zoonosis in *C. difficile* disease, identify the contribution of spores in the human food chain, and collect isolates from human CA-CDAD cases.
- Improve and utilize clinical scoring systems early in the progression of disease to predict persons with potentially poor outcomes, those who will fail metronidazole therapy and other elements. Validate the clinical scoring system prospectively.
- Develop a solid and sensitive toxin assay in blood or serum to measure organs other than the colon that play a role in severe *C. difficile* disease and toxicity.
- Partner with the American Institute of Architects of Healthcare Design in designing spaces in hospitals to increase hand washing among healthcare providers.

Summary of Key Findings

Dr. John Bartlett is the Chief of the Division of Infectious Diseases at John Hopkins University. His summary of the key findings and recommendations made during the workshop is outlined below.

- Perform research on overlapping areas between *C. difficile* and *C. sordellii*, such as the regulation and mechanism of toxins, the role of sporulation, virulence factors, and the role of flora in controlling the organism.
- Conduct sentinel studies to collect more data on *C. difficile*, particularly in the areas of non-antibiotic use and the new epidemic strain.
- Use the tissue culture assay and common antigen as a screening test until the rapid, inexpensive and sensitive PCR technology that will identify toxin B is released in the near future. Advance efforts to release the NAP1 test that will provide information about the new strain.
- Increase the practice of culturing stool in the United States or develop and use binary toxin, deletion or another non-culture-based method to recognize *C. difficile* strains.
- Clearly determine whether vancomycin or metronidazole should be used to treat *C. difficile*. Clearly distinguish between agents that are needed to treat acute versus intermittent disease. Acknowledge that vancomycin is viewed as the "perfect drug" in treating acute disease.
- Focus new drug development on intermittent and recurrent *C. difficile* disease for patients who are unable to take oral therapy due to the significant morbidity in this population.
- Review cases of CA-CDAD and antibiotic exposures to determine the number of cases hospitals were justified in using antibiotics.
- Develop guidelines on the use of drugs that do not drive *C. difficile* disease. Highlight the significant differences among the broad-spectrum clindamycin, cephalosporin and fluoroquinolone classes of drugs.
- Launch a national initiative to add HA-CDAD as a reportable disease.
- Strongly urge public health to partner with the VA because this agency maintains the best and largest electronic medical record system in the world. Utilize this valuable resource to obtain rapid answers on *C. difficile* from data that were previously collected.
- Improve surveillance systems of *C. sordellii* to increase capacity in detecting the infection.

- Revitalize laboratory interest in the area of anaerobic cultures to enhance capacity to rapidly and inexpensively culture *C. difficile*, *C. sordellii* or other histotoxic *Clostridia*.

Closing Session

Dr. Leslye Johnson is the Chief of the Enteric and Hepatic Diseases Branch at the NIH National Institute of Allergy and Infectious Diseases. She made the following remarks to conclude the workshop. The expert panel and members of the audience are to be commended in providing CDC, FDA and NIH with up-to-date findings, interesting hypotheses, valuable recommendations, and solid future directions in developing a research agenda for complex issues related to *C. difficile* and *C. sordellii*. The research agenda will serve as a living document that will be modified over time as new science is produced.

The organisms emphasize the critical need for multi-disciplinary research. Most notably, NIH encourages investigators to apply for R01 grants and funding for small developmental studies because *C. difficile* and *C. sordellii* are now viewed as priorities throughout the agency. Federal attention will continue to be paid to these issues through CDC's external partnerships and ongoing interagency collaborations among CDC, FDA and NIH.

Dr. Johnson confirmed that NIH is extremely encouraged about clinical trials for *C. difficile* that are underway to identify effective therapeutic and preventive approaches. She emphasized that the ongoing research will provide an opportunity for public/private partnerships between NIH and other federal agencies to advance the field as quickly as possible.