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Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

SESSION X—CLOSTRIDIUM DIFFICILE: MANAGEMENT

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

CURRENT AND FUTURE TREATMENT OPTIONS FOR *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)

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Treatment of *Clostridium difficile* (CDI) infection remains a clinical challenge due to declining response rates to metronidazole, continued high rates of recurrent CDI, and inadequate management strategies for fulminant or severe complicated CDI.

Review of the current state of treatment for first episodes of CDI indicates that stratification of patients into mild or moderate vs. severe disease is critical to determination of what the best initial treatment agent should be. Two randomized, prospective, and blinded trials have shown that oral vancomycin is superior to metronidazole for treatment of severe CDI, but that there is no statistically significant difference between metronidazole and vancomycin for treatment of mild to moderate CDI. The major unanswered question is how to determine CDI severity as there have been no validated scoring systems published. At this time, elevated peripheral WBC of >15,000-20,000/mm^3 appears to be one criteria on which to base severity. Other indicators include presence of pseudomembranous colitis, need for ICU care, hypotension, elevated creatinine, fever, degree of abdominal pain or tenderness, number of stools per day, and CT findings of thickened colonica wall and ascites. New therapies under development are approaching the treatment dilemma from several directions including use of narrow spectrum non-absorbable antibiotics, such as OPT-80, use of non-antibiotic toxin binding strategies, such as polymers (tolevamer); or milk derived antibodies designed to allow recovery of the normal colonic flora while treating CDI to reduce recurrence. Still, another approach is biotherapeutic, using live bacterial organisms to restore the protective flora and include fecal transplants, *Saccharomyces boulardii* and other probiotics, and non-toxigenic *C. difficile*. An additional approach is to increase antibody response to *C. difficile* and its toxins through use of vaccines or monoclonal antibodies which could reduce disease rates overall and possibly improve treatment of fulminant disease through passive immunity. Results to date have been somewhat discouraging for new products, but multiple new agents are under investigation.

It is currently unclear what strategy (narrow spectrum antibiotic, biotherapeutic, toxin binding, or vaccine/antibody) will be most successful in managing CDI, but it is clear that both preventive and therapeutic interventions are critically needed to reduce incidence and morbidity/mortality from CDI, particularly among elderly hospitalized patients.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

FECAL BACTERIOTHERAPY FOR RECURRENT *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA

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Clostridium difficile-associated diarrhea (CDAD) has emerged as a major complication associated with the use of systemic antimicrobial agents. Broad-spectrum antimicrobial agents disrupt the ecological bacterial balance in the colon and create an opportunity for *C. difficile* overgrowth with attendant production of toxins and clinical symptoms of colitis. Recommended therapies for CDAD include oral administration of metronidazole or vancomycin for 10 to 14 days. However, both metronidazole and vancomycin may perpetuate the collateral damage to the resident bacterial flora in the colon, and 5% to 50% of patients experience infection relapse after completion of treatment. Furthermore, the relative risk for subsequent relapses increases with each successive treatment course. Recently, patients who failed to resolve their infection with conventional therapies and went on to develop chronic relapsing, CDAD were successfully treated with fecal bacteriotherapy. Stool obtained from a healthy individual was instilled from either end of the GI tract (via an NG tube or a colonoscope, respectively). Once the stool had been instilled, bacterial homeostasis in the colon was quickly restored, which resolved the relapsing diarrhea pattern. Although the published experience with fecal bacteriotherapy is still limited, the published treatment results for more than 80 patients have demonstrated an average success-rate higher than 90%. Fecal bacteriotherapy is a low tech procedure, which is easy to perform, and breaks the cycles of repeated antibiotic use, which in turn reduces the risk of antibiotic associated resistance and adds potential cost savings, when compared to repeated antibiotic administration and hospitalizations.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

C245T (Thr82Ile) MUTATION IN *gyrA* OF *CLOSTRIDIUM DIFFICILE* BI EPIDEMIC ISOLATES HIGHLY RESISTANT TO MOXIFLOXACILLIN

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To characterize the nature of moxifloxacin resistance among *C. difficile* isolates recovered during a BI-associated, nosocomial outbreak of antibiotic associated diarrhea, we measured the susceptibility of 34 field and 6 laboratory isolates to moxifloxacin and genotyped each by PCR of their *tcdA, tcdB, tcdC, gdh, cdtA* and *cdtB*. The field isolates were collected in 2001 and 2002 during a nosocomial outbreak of antibiotic associated diarrhea in Pittsburgh and included BI as well as non-BI isolates (McEllistrem et al., 2005, Clin. Infect. Dis., 14:265-72). All the laboratory isolates, including Cd196, a BI isolate, were susceptible to moxifloxacin (2 μg/mL). 13 field isolates were susceptible to 2 μg/mL. Five were susceptible to 4 to 12 μg/mL (low resistance); 16 were resistant to 16 μg/mL (high resistance). We sequenced the quinolone resistance determining regions of *gyrA* (position 71-460) and *gyrB* (position 1059-1448) from two susceptible controls, all 5 isolates with low resistance and 2 highly resistant isolates. The highly resistant isolates (one a BI 9, the other a J 9 with a wild type PCR genotype) had the same C245T (Thr82 Ile) mutation seen by others, though not before now in BI isolates. No other changes were seen. Two PCR primer pairs specific for the C245T mutant *gyrA* and for the wild type respectively, showed all 16 highly resistant isolates, BI as well as non-BI, had the C245T mutation and that mutation was absent from all other isolates. Among the 5 isolates with low resistance, we found combinations of mutations within *gyrA* (T128A, Val43Asp and G349T, Ala117Asp) and *gyrB* (G1276A, Arg447Leu and G1429A, Glu466Lys). Some of these are novel. In summary, during the outbreak, not all highly resistant isolates were BI isolates, furthermore BI isolates were not always resistant and, lastly, a variety of mutations likely to confer resistance were found among the broader range of isolates, though in resistant BI isolates, the mechanism of resistance was identical.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

REP3123, A NOVEL INHIBITOR OF METHIONYL tRNA SYNTHETASE (MetRS) FROM *CLOSTRIDIUM DIFFICILE*

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Purpose: *C. difficile* associated disease (CDAD) is caused by overgrowth of toxin-producing strains of *C. difficile* (CD) following disruption of normal gut flora. Currently, there are few therapeutic options, and clinical problems are associated with disease recurrence and the persistence of spores in the environment. In this report, we describe biochemical, crystallographic, microbiological and *in vivo* efficacy properties of REP3123, a novel diaryldiamine inhibitor of CD MetRS.

Methods and Results: REP3123 was synthesized and purified as a single active enantiomer. The enzyme was crystallized by sitting drop vapor diffusion and structures were solved by molecular replacement. Microbiological testing was done according to CLSI guidelines. Toxins A and B were detected by semi-quantitative immunoassays and cytotoxicity assays. *In vivo* efficacy of REP3123 was evaluated in the hamster model of CDAD.

The tight binding of REP3123 to MetRS (K_i=20 pM) is competitive with methionine but cooperative with ATP. Inhibitor binding is accompanied with a conformational change in the enzyme in which the inhibitor occupies the methionine binding pocket and a newly created hydrophobic pocket. REP3123 is highly active against CD, including the NAP1/027 outbreak strain (MIC range, 0.25-1.0 mg/L). In contrast, REP3123 is inactive against Gram-negative bacteria and most anaerobic bacteria that constitute normal gut flora such as *Actinomyces, Bacteroides, Bifidobacterium* and *Lactobacillus* species. As a protein synthesis inhibitor, REP3123 inhibits the production of CD toxins A and B under a variety of culture conditions. REP3123 is also highly effective in inhibiting spore formation. *In vivo*, REP3123 exhibits low oral bioavailability and superior efficacy to vancomycin in the hamster model of CDAD.

Conclusion: REP3123 is a novel mechanism-of-action antibacterial agent that exhibits potent and selective inhibition of CD with limited potential for disruption of normal gut flora. REP3123 effectively inhibits toxin production and sporulation, exhibits low oral bioavailability and protects against CDAD *in vivo*. With these unique features, REP3123 represents a promising new development candidate for the treatment of CDAD.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

CLOSTRIDIUM DIFFICILE-ASSOCIATED DIARRHEA (CDAD) IN A LONG-TERM ACUTE CARE FACILITY (LTAC)

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Background: *Clostridium difficile*-associated diarrhea (CDAD) is the 2nd most common nosocomial infection in hospitals and impacts increased hospital cost and length of stay. Most surveys have looked at incidence and risk factors for its development. Since scant information is available about the incidence and prevalence of CDAD in LTACS, we therefore studied this at one local facility.

Methods: Demographic data, prior antimicrobial exposure, and a fresh stool sample were obtained from all new LTAC admissions not carrying a prior diagnosis of CDAD during study period (7-23 to 8-22-07). An ELISA test for antigen [C. diff quick chek, TechLab] was performed. All initially positive stools were tested for toxin A and B and a sample frozen for isolation and typing. All antigen-negative patients were monitored for the development of diarrhea during the course of their LTAC hospitalization and, if clinically indicated, a sample was sent for toxin A and B testing and if positive a stool sample was frozen and stored for isolation and typing. Therapy of CDAD was noted.

Results: 36 patients were admitted during the study period. 4 of 31 (12.9%) of patients were antigen (+) on admission of which 2 (6.5%) had unsuspected active disease including one with the BI epidemic strain. In follow-up, 20/36 (55.5%) developed diarrhea of which an additional 3 (8.8%) patients subsequently developed CDAD in the hospital. During that annual quarter the rate of nosocomial acquired CDAD was 3.12 per 1,000 patient days

Conclusions: *C. difficile* carriage and unsuspected clinical CDAD occurs, including the BI epidemic strain disease, in an important minority of patients, which may act as a reservoir for spread. New strategies for detection and prevention of CDAD are needed

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

IN VITRO ASSESSMENT OF SUSCEPTIBILITY OF 163 *CLOSTRIDIUM DIFFICILE* ISOLATES TO RIFAXIMIN

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Purpose. This in vitro study analyzed *Clostridium difficile* isolates collected from a single center to identify hypervirulent strains, evaluate susceptibility to rifaximin, and assess the value of rifampin E strip testing for predicting *C. difficile* resistance to rifaximin.

Methods. *C. difficile* isolates were obtained between 2006 and 2007 from consecutive patients treated for hospital-acquired diarrhea whose stools were positive for *C. difficile* cytotoxin B, determined via tissue culture assay. Stools were cultured on *C. difficile*–selective agar (CCDA) with isolates and analyzed by PCR techniques for toxins A and B, binary toxin (BT), and partial deletions in the *tcdC* gene (*tcdC*-del). *C. difficile* susceptibility to rifampin was determined with rifampin E strips, and susceptibility to rifaximin was assessed using an agar dilution method recommended by the Clinical and Laboratory Standards Institute. Resistance was defined as a minimal inhibitory concentration (MIC) >32 μg/mL for rifampin and rifaximin.

Results. A total of 163 *C. difficile* isolates, including 32 positive for BT and *tcdC*-del, were analyzed. For rifaximin, MIC_{50} was <0.01 μg/mL and MIC_{90} was 0.125 μg/mL. Rifampin had MIC_{50} <0.002 μg/mL and MIC_{90} of 0.75 μg/mL. Of 163 isolates analyzed, 12 (7%) were resistant to rifampin, of which 2 (1% of 163) were resistant to rifaximin. Of the 12 isolates resistant to rifampin, 2 were A/B/BT/*tcdC*-del positive, 1 was A/B/BT positive, 8 were A/B positive, and 1 had no detectable toxin genes. Of the 2 isolates resistant to rifaximin, 1 was A/B/BT positive and 1 was A/B positive without BT/*tcdC*-del.

Conclusions. These findings confirm previous reports of high rifaximin activity against *C difficile* and suggest that, although resistance of recent *C. difficile* isolates to rifaximin may occur in vitro, the incidence is low (1%). To date, rifaximin resistance in isolates with *tcdC* deletions has not been observed. Furthermore, susceptibility testing with rifampin E strips did not predict *C. difficile* resistance to rifaximin in the hospital population studied.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

SUBSEQUENT EXPERIENCE USING RIFAXIMIN AS A POST-VANCOMYCIN TREATMENT STRATEGY FOR RECURRENT *CLOSTRIDIUM DIFFICILE* DIARRHEA

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We previously reported our experience using rifaximin as a post-treatment strategy (the rifaximin 'chaser') in 7 patients with multiple recurrences of *C. difficile*-associated diarrhea (CDAD) (Johnson S et al., Anaerobe 2006/Clin Infect Dis 2007;44:846-848). Only 1 of those 7 patients had a subsequent CDAD episode and that patient responded to a second course of rifaximin. We now report results in the next 5 patients with multiple CDAD recurrences from our clinic in which this strategy was used.

The ages of these 5 patients ranged from 33 to 85 years (mean age ± SD= 73 ± 22.7; median age= 81) and 4 of the 5 were female. Each patient had between 3 and 8 prior CDAD episodes (mean episodes ± SD= 5.4 ± 2.1). The regimen used to unsuccessfully treat the last episode prior to the rifaximin chaser regimen in each case was a tapering or tapering/pulsed vancomycin regimen. Following that recurrence, the patients were treated with vancomycin 4 times daily until they were asymptomatic at which time the vancomycin was stopped and rifaximin (400 mg BID) was given for 2 weeks. Three of the 5 patients had no further diarrhea recurrence (follow up at least 2.5 months for each), but 2 patients had recurrent CDAD episodes after completion of or during rifaximin treatment. The first patient recurred 3 days after completing the rifaximin regimen; She was then treated with vancomycin until asymptomatic and then given rifaximin again with the plan to continue treatment for a longer course, but 3 days into the second course her stools became soft and by day 10, she developed frank diarrhea. *C. difficile* was recovered from stool specimens obtained at the time of both rifaximin failures and the isolates in each case had rifaximin MICs of > 256 ug/ml. In retrospect, rifaximin had been used as a treatment agent for an earlier episode of CDAD in this patient. The second patient developed fever and diarrhea after 4 days on rifaximin and was re-hospitalized 24 hours later because of dehydration. The stool specimen from this patient at the time of recurrence was toxin-negative, but *C. difficile* was recovered from culture; The MIC of this isolate to rifampin, however, was ≤ .015 ug/ml.

In summary, interruption of multiple CDAD recurrences with the post-treatment rifaximin strategy was not as effective as in our initial experience, but the failures were recognized during or immediately after the rifaximin course and did not consistently demonstrate *C. difficile* resistance to rifaximin. In contrast, 3 of the 5 (60%) patients recovered from this devastating and prolonged manifestation of *C. difficile* disease. More studies comparing treatment outcome and in vitro susceptibility to rifaximin are needed.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

THE COMMERCIALLY AVAILABLE RIFAMPIN E-TEST PREDICTS RIFAXIMIN SUSCEPTIBILITY IN CLINICAL ISOLATES OF *CLOSTRIDIUM DIFFICILE*

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Background: Rifaximin, a poorly absorbed rifamycin derivative, is undergoing clinical trials for treatment of *Clostridium difficile* infection. However, reports of *C. difficile* strains with high rifaximin MICs exist. Rifamycin resistance in bacteria traditionally occurs due to point mutations in RpoB, the β-subunit of RNA polymerase but it is not known whether this also occurs in *C. difficile*. The prevalence of rifaximin resistance in current *C. difficile* isolates and the clonality of such isolates are unknown. There is no commercially available test for rifaximin susceptibility, but a commercial E-test for the related agent, rifampin, exists. **Methods:** Eighty unique *C. difficile* clinical isolates were subjected to three different analyses: agar dilution susceptibility testing (rifaximin and rifampin) and E-test analysis (rifampin). Isolates with a Minimum Inhibitory Concentration (MIC) ≥32 μg/ml for both compounds were considered to be resistant. All isolates were typed using Restriction Endonuclease Analysis (REA) and their geographic origin was recorded. Finally, the *rpoB* sequences of five sensitive and fourteen resistant isolates were compared to identify specific sequence changes associated with rifamycin resistance.

Results*:* Rifaximin and rifampin susceptibility testing revealed a bi-modal MIC distribution. Strains either had very high (≥ 32 μg/ml) or very low (≤1μg/ml) MICs by all three methods of analysis. Fourteen isolates were resistant to both rifaximin and rifampin and molecular typing revealed that these isolates belonged to five distinct REA groups. Further, seven different RpoB sequences were associated with rifamycin resistance. The combined data from REA, geographic origin analysis and *rpoB* sequencing demonstrated that at least 5/14 resistant isolates were independently derived. A further 5/14 resistant isolates were also likely to be independent as they were from geographically distinct locations but were similar at the molecular level.

Conclusions: The rifampin E-test can predict rifaximin resistance in *C. difficile*. The incidence of rifamycin resistance in *C. difficile* may be greater than previously suspected. We propose that selective pressure is responsible for the detection of *C. difficile* rifamycin resistance. Correlation of clinical outcome and isolate susceptibility testing after rifaximin treatment will be important for future trials.

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CLOSTRIDIUM DIFFICILE: MANAGEMENT

CLOSTRIDIUM DIFFICILE BACTEREMIA: A CASE REPORT

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Introduction: *Clostridium difficile*, a ubiquitous anaerobic gram-positive rod responsible for antibiotic-associated diarrhea and pseudomembranous colitis, is increasingly becoming a major nosocomial pathogen. Several states have had recent outbreaks with an epidemic strain of *C. difficile*, and health care facilities are reporting alarming rates of *C. difficile* infections. In contrast, reports of *C. difficile* infections in extraintestinal sites have historically been infrequent and anecdotal. We report a unique case of *C. difficile* bacteremia in a non-neutropenic host.

Case Presentation: A 59-year-old man with a history of chronic obstructive pulmonary disease, upper gastrointestinal bleeding, prostate cancer, bilateral deep vein thrombosis, hydronephrosis with ureteral stent, and chronic kidney disease was admitted to Our Lady of Medical Center, NYMC (Bronx, NY) for evaluation of hematuria. On initial evaluation, blood pressure was 124/87mmHg and patient was hypothermic. Abdominal exam was significant for mild distension with normoactive bowel sounds. Laboratory evaluation: WBC count of 8,600/mm^3; creatinine of 5.5mg/dL. Urinalysis: WBC 50-100 with packed RBCs and positive nitrite. Chest X-ray: bilateral pleural effusions and bilateral lower lobe consolidations. Patient was started on moxifloxacin. Stool for *C. difficile* toxin assay was sent as patient had loose stools and a history of hospitalization 3 weeks prior. *C. difficile* toxin assays came back positive on the second and third hospital days. Patient was started on metronidazole, and moxifloxacin was changed to cefepime. On the third day, initial blood cultures reported growth of gram-positive rod in anaerobic bottle. Urine cultures grew *Enterococcus* species. Despite treatment, hypothermia continued and his clinical status worsened. On the 6^{th} day, blood cultures grew *C. difficile* (identified by RapID ANA-II System and later confirmed by another laboratory). Patient was treated with oral and intravenous vancomycin (1 gram post dialysis). On the ninth hospital stay, patient expired.

Discussion: *C. difficile* is a spore-forming organism, found in the environment and colonizes 3-5% of normal adult human beings. Exposure to antibiotics has been associated with proliferation of this organism. Despite the frequent encounter of individuals with *C. difficile* associated diarrhea (CDAD) and colitis, severe complications are unusual. Isolation of *C. difficile* from extraintestinal sites —such as trauma sites, abscesses, or body fluids is extremely uncommon and often polymicrobial in nature. *C. difficile* bacteremia have rarely been reported in the literature with only 3 cases in the past 20 years.

In an era of increasing *C. difficile* infections, along with greater virulence especially with the epidemic strains, more complications are expected. *C. difficile* bacteremia is associated with high mortality. We should be vigilant and recognize atypical extraintestinal presentations, including sepsis and bacteremia, so we can treat them quickly and appropriately.