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SESSION IX: CLOSTRIDIUM DIFFICILE: TREATMENT OPTIONS

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CLOSTRIDIUM DIFFICILE: TREATMENT OPTIONS

NARROW SPECTRUM THERAPY OF *CLOSTRIDIUM DIFFICILE* INFECTION

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Clostridium difficile infection is recognized as a prime example of disease as a result of disturbance of the host intestinal microflora. However the degree of impairment of the microbiome at the time of disease presentation, effects of metronidazole or vancomycin on both the pathogen and normal flora components during therapy, and microbial floral shifts follow therapy when patients are prone to recurrence of disease are still not well understood. The opportunity to examine the foregoing issues arose during the conduct of recent randomized clinical trials to evaluate new treatments for CDI with the view to more directed or narrow spectrum therapy of CDI. Vancomycin and metrondazole were compared to tolevamer, a toxin A and B binding resin. Fidaxomicin, formerly OPT-80, was evaluated as a narrower spectrum agent on the basis of high activity versus the pathogen but poor activity against gram negative obligate anaerobic organisms in-vitro. These studies served as quasi biological probes on the importance of the components of the normal microbiome in the pathogenesis of CDI. These trials illustrate that the best strategy for treatment is to simultaneously kill the pathogen / negate the effects of toxin and to allow reconstitution of the normal microbiome, a cornerstone for prevention of recurrent disease. Results of ecologic studies done during the clinical trials will be summarized.

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"OUTSIDE THE BOX" MANAGEMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION: MONOCLONAL ANTIBODIES, VACCINES, AND NON-TOXIGENIC *C. DIFFICILE*

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Clostridium difficile infection (CDI) is precipitated by antimicrobial use in the vast majority of cases and is also treated with antimicrobials ("inside the box" management) that perpetuate the risk of recurrent CDI by their unintended suppression of the protective normal gut microbiota. Susceptibility to CDI is postulated to be a result of a combination of suppressed normal gut bacteria and a deficiency in the immune (antibody) response to *C. difficile* toxins. Management (prevention and treatment) of CDI by non-antimicrobial means could result in fewer primary and recurrent cases. Three such "outside the box" approaches are currently undergoing clinical evaluation in human subjects; passive administration of monoclonal antibodies (Mabs), development of an injectable *C. difficile* vaccine, and re-establishment of the protective effect of the flora using non-toxigenic *C. difficile* (NTCD).

A phase II trial of the use of Mabs directed at toxin A and at toxin B in 200-patients has shown that the intravenous Mabs, when given to CDI patients in conjunction with standard metronidazole or vancomycin treatment, reduced CDI recurrence from 25% to 6.9% compared to placebo (P=.0004). In contrast, administration of a single Mab directed at toxin A did not show a benefit.

An active vaccine approach (ACAM-CDIFF) utilizing inactivated whole toxins A and B given as three intramuscular injections at various doses and dosing schedules has been administered to >200 volunteers including those ≥65 years of age and has been shown to be immunogenic and safe. The vaccine is currently being tested with and without adjuvants on a schedule of day 0, 7, and 28 in patients with CDI to establish benefit in reducing CDI recurrence. Evidence of efficacy is limited to treatment of 3 patients with recurrent CDI, all of whom demonstrated an immune response and cessation of further CDI.

NTCD (VP20621) is a preparation of spores that has been administered as a suspension to 42 volunteers (27 were ≥60 years old) at doses of 10^4, 10^6, and 10^8 spores given once or twice daily for 1 to 5 days. All doses were well tolerated compared to placebo patients. In patients who received 10^8 spores twice daily, NTCD was detected in stool cultures of 7/12 asymptomatic patients on day 3 and 6/12 on day 7 but none on days 14 or 21. Detection of NTCD in stools most likely represents "pass-through" in these volunteers. Evaluation of volunteers after pre-treatment with antimicrobials is in process.

These "out of the box" strategies show high promise for improved CDI management in the future.

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ANTIBODIES TO *CLOSTRIDIUM DIFFICILE* IN PATIENTS DURING TREATMENT WITH METRONIDAZOLE, VANCOMYCIN, OR TOLEVAMER

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Phase 2 trials of Tolevamer (TOL) a toxin-binding agent for patients with *C. difficile* infection demonstrated that those treated with the agent had fewer relapses compared to those treated with vancomycin (VAN) or metronidazole (MET). This suggested that TOL may have immunomodulatory activity enhancing the immune response and protecting from relapse. An opportunity to test this hypothesis arose during Phase 3 trials where a sub-study was set up to measure antibody levels during treatment. The primary aim was to test this hypothesis, with an important secondary aim to study the kinetics of antibodies to toxins and cell-surface antigens throughout the course of disease, relating response to outcome.

From 11 sites in Europe and N America a total of 34 patients had blood taken on 4 occasions: day 1 and 4 of treatment, day 15 after end of treatment and at follow-up around day 30. TOL was used in 16 patients and VAN or MET in 9 each. In this sub-study the groups were well matched for age: mean age of TOL group 57.8, VAN 52.4 and MET 52.1 years; and disease severity, severe: moderate: mild; TOL 4:3:9; VAN 1:4:4 and MET 2:3:4. A successful outcome was achieved in 8/16 (50%) of cases in the TOL group, 7/9 (78%) in the VAN group and 6/9 (67%) in the MET group – reflecting the results of the full trial. Overall the numbers of patients not suffering relapses was 7/16 (44%) in the TOL group, compared to 6/9 (67%) in the VAN group and 6/9 (67%) in the MET group.

ELISA was used to study the kinetics of responses to 5 antigens: (i) pure Toxin A, (ii) crude toxins (incl. extracellular antigens), (iii) S-layer proteins, (iv) membrane lipocarbohydrate and (v) a cell-surface complex.

At the start of treatment antibody levels to antigens varied, from 3-fold less to 4-fold more than a standard (serum pooled from 250 blood donors). Kinetics were similar in each treatment group with mean levels increasing insignificantly over time. When treatment groups were combined responses to all 5 antigens could be analysed in 28 patients. Symptoms resolved in 19 of these with 3 subsequently relapsing. All patients whose symptoms resolved had high antibody levels to 1-5 antigens (mean 2.66/5 antigens) on day 1. Subsequently 14 of these patients showed a marked rise in antibody levels to an overall mean of 2.0/5 antigens. Of the 5 patients showing no increase in antibodies 2 relapsed. These had low day-1 levels, as did the other relapsing patient. The 9 patients failing to respond to treatment had much lower levels of antibody on day 1 (a mean of 1.5/5 antigens compared to standard) and no rise was seen in 5 patients, giving an overall mean rise to 0.7/5 antigens.

This pilot study suggests that high levels of antibody at the start of treatment, together with the ability to produce an antibody response, were positive indicators for successful treatment. However, there was no evidence for Tolevamer enhancing the immune response.

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FIDAXOMICIN AND VANCOMYCIN POSTANTIBIOTIC EFFECT AGAINST *C. DIFFICILE*

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Fidaxomicin (FDX) (formerly OPT-80 and PAR-101) is the first in a new class of narrow spectrum macrocyclic antibiotic drugs that is being developed for the treatment of *Clostridium difficile* infections (CDI). In a recent Phase 3 clinical trial, FDX demonstrated significantly lower recurrence ($p = 0.004$) and better global cure rate ($p = 0.006$) compared to vancomycin. The clinical success may be attributed to targeted bactericidal activity against *C. difficile* and minimal disruption of the gut flora. Another positive attribute is the reported long postantibiotic effect. In this study, we reevaluated the FDX PAE to further demonstrate that the long recovery rate is not an artifact of residual drug that may have nonspecifically adsorbed to the plastic surfaces during the experiment.

The PAE of FDX and vancomycin was measured by recovery kinetic curves after one hour of exposure of ATCC *C. difficile* strains (9689 and 43255) to drugs at 4xMIC. To eliminate any potential artifact due to nonspecific binding of drug, following drug removal, the cells were transferred to a new tube and their recovery rate was compared to the cells that remained in the original tube. The PAE was determined as the time required for the titer to increase one log (i.e. 10-fold) over the post-washing titer.

FDX was found to have a prolonged PAE, an average 10 hrs with the 2 ATCC strains. This result was reproduced in duplicate experiments and the results did not vary more than 2.5 hrs; PAE for strain 9689 was at 9.5 and 10 hrs and for strain 43255 at 9 and 11.5 hrs. The long recovery rates reported earlier most probably represented the postantibiotic sub-MIC effect (PAE-SME); contributed by potential non-specific binding of drug to plastic surfaces. Vancomycin at 4xMIC, used as a control, with the same method, performed as previously reported with a PAE of 0 - 1.5 hrs.

The findings of this study confirm that FDX PAE is prolonged. Notably it is much longer than that of comparator drug vancomycin (an average of 10 hrs vs. 0 – 1.5 hrs, respectively). Prolonged PAE and prolonged PA-SME could be advantageous in a severe diarrhea condition like CDI in which drugs are administered 2 or 4 times per day but the rapid transit time in the bowel could eliminate the drug in the bowel before the next dose. The residual PAE would provide antimicrobial activity against *C. difficile* even in the absence of drug levels. In addition, a prolonged PAE may allow for reduced dosing of FDX without impairing efficacy.

Fidaxomicin, FDX, OPT-80, *C. difficile*, *C. difficile* infections, Postantibiotic effect

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CLOSTRIDIUM DIFFICILE DIARRHEA: SHOULD EVERY RELAPSE BE TREATED?

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Two young adults (P1 and P2) with community-acquired *C. difficile* diarrhea were monitored during diarrhea, therapy, relapse and recovery. We tested for toxin A and toxin B, glutamate dehydrogenase (GDH), and lactoferrin, a marker of inflammation. We grew *C. difficile*, ribotyped isolates, tested them for toxin production, and by PCR for virulence genes. About 6 d after 48h of vomiting and diarrhea P1 presented with mild, non-antibiotic associated *C. difficile* diarrhea. Metronidazole (MZ) was rapidly effective; symptom and laboratory findings returned to normal within 2 or 3 days. 7 d after treatment P1 relapsed. Compared with the original symptoms, her pain was greater and she passed more stools per day. Her feces were more watery and now contained mucous. MZ was again rapidly but temporarily effective. A 2nd relapse followed within 10 d. The initial diarrhea and 1st relapse were caused by 027 isolates, the 3rd by a ribotype TL5019 isolate. Fecal levels of toxins A and B, and GDH could not distinguish between episodes, nor were total or sporulating *C. difficile* counts discriminatory. Fecal lactoferrin though, was much higher during the 1st episode (812 µg/g feces) than in episodes 2 and 3 (402 and 316 µg/g respectively). This finding, and the self-reported mildness of symptoms during episode 3, and the observation made during episodes 1 and 2 that MZ quickly reduced evidence of inflammation and other symptoms, encouraged a more cautious attitude in P1 and in her physician to further MZ therapy. Before symptoms warranted initiating MZ, the immuno-analytes, notably fecal lactoferrin, began to decline and the symptoms to abate. P1 made a full recovery without further therapy. Patient 2 had an initial episode of antibiotic associated *C. difficile* ribotype 027 diarrhea with heavy cramping. Sample collection began after MZ was started, by when fecal lactoferrin was only 61 µg/g. MZ therapy preceded a ribotype 027 relapse with only mild cramping and 200 µg/g of fecal lactoferrin. A 2nd spell of MZ was started. There followed 6 weeks of asymptomatic colonization by ribotype 027 during which toxin A, toxin B, GDH and lactoferrin were consistently absent from P2's feces. This culminated in 3 d of continued colonization associated now with the presence of toxins A and b and GDH but still not lactoferrin. P2 chose not to take a 3rd round of MZ; symptoms did not develop and laboratory findings resolved without resort to therapy. Both P1 and P2 are healthy today. We conclude that not all presentations of *C. difficile* warrant immediate treatment. Shifts in fecal lactoferrin level may provide clues about those that do.

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ANTIMICROBIAL PROFILE OF *CLOSTRIDIUM DIFFICILE* AND *IN VITRO* ACTIVITY OF *GARCINIA KOLA* EXTRACT

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In recent years, several reports on the increasing rise of resistant *Clostridium difficile* with reduced susceptibility to drugs of choice, metronidazole and vancomycin have been reported. This has caused both the clinicians and researchers alike to search for viable and affordable therapeutic alternatives. Although the impacts of *C. difficile* infections from the western countries have been widely reported, there is however, paucity of information on *C. difficile* infections in Nigeria where there is little or no restrictions on the use of antibiotics. We investigated the antimicrobial susceptibility pattern of clinical strains of *C. difficile* and compared it to the inhibitory effect of a local plant extract *Garcinia kola*, a plant currently used in popular medicine in Nigeria and where the nuts of this plant are usually chewed as snacks. A total of thirty eight strains of *C. difficile* were tested for their susceptibility to eight antibiotics, the methanol and aqueous extracts of *G. kola* by the CLSI agar dilution method. The plate with the lowest concentration on which there was no evidence of growth was taken as the minimum inhibitory concentration (MIC). All strains were susceptible to vancomycin and metronidazole, while 54.5% were susceptible to ceftriaxone. High resistance to other antibiotics such as rifampicin (99.5%), ciprofloxacin (98.5%), amoxicillin (91.2%), clindamycin (86.4%) and cefotaxime (72.7%) was observed. The seed of *G. kola* demonstrated variable significant inhibitory effect on all *C. difficile* isolates. The average MIC of the aqueous extract for all isolates was 12.5mg/ml and 3.215mg/ml for the methanol extract. This study shows that crude extracts of *G. kola* possess antibacterial activity and may have a potential role as an active biological substance for new drugs against *C. difficile* infections. However, there is need for future studies to identify the active agents and determine the effect of the extract on human cells.

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CLOSTRIDIUM DIFFICILE: TREATMENT OPTIONS

ACAM-CDIFF™: AN ACTIVE VACCINE AGAINST *CLOSTRIDIUM DIFFICILE* INFECTION

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In the past, *Clostridium difficile* infection (CDI) had been considered a manageable nuisance infection associated with hospitalization. In recent years, due to the changes in epidemiology and demographics as well as increasing costs associated with this infection, *C. difficile* has become a major management challenge in institutional settings and, accordingly, a target for new interventions. A primary risk factor for CDI is prior antibiotic therapy and yet, despite this, the current standard of care involves a choice of two antibiotics. In light of this paradox and the growing appreciation of the role of gut microbial ecology in preventing opportunistic infections, non-antibiotic approaches are gaining favor. Of these, immune-based strategies are promising, given the role of natural immunity in suppressing disease symptoms in individuals with robust immune systems and the effectiveness of passive administration of *C. difficile*-directed antibodies. Because current CDI therapy does not preclude disease transmission in institutional settings, prevention as a key management approach is warranted.

An active vaccine directed against *C. difficile* toxins (ACAM-CDIFF™) shows promise as an effective approach for primary prevention of CDI with the potential to also prevent recurrent CDI episodes. As the role of toxin B in human disease becomes further clarified, it is now apparent that a vaccine comprised of inactivated toxins A&B has the greatest likelihood of success. Furthermore, as our understanding of the patient immune response to CDI improves, there is growing confidence that protective immunity can be elicited with the vaccine.

The ACAM-CDIFF™ vaccine is in Phase II clinical development with studies addressing the primary prevention and prevention of recurrence CDI indications planned or ongoing. The prospects and challenges for a preventative vaccine against CDI will be presented.

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CLOSTRIDIUM DIFFICILE: TREATMENT OPTIONS

IN VIVO EFFICACY OF ORITAVANCIN IN THE HAMSTER MODEL OF *CLOSTRIDIUM DIFFICILE* INFECTION

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Background: Oritavancin (ORI) is a lipoglycopeptide with activity against *Clostridium difficile* (CD). It inhibits CD spore outgrowth *in vitro* and in a gut model of CD infection (CDI) it has minimal impact on normal gut flora. We report here the pharmacokinetic (PK) profile and the efficacy of ORI in a hamster model of CDI.
Methods: Golden Syrian hamsters were injected subcutaneously with clindamycin (CL) (100 mg/kg) one day before CDI (on Day -1) followed by gavage with 10^5 CFU of spores of CD ATCC 43255 on Day 0. On Day 1, ORI (10, 50 or 100 mg/kg) formulated in HPCD, vancomycin (VA) in PBS (50 mg/kg) (n=10 hamsters/group), or ORI formulated in PEG400 (100 mg/kg) (n=5/group) were administered orally (PO) once daily for 5 days. Animals were monitored for clinical signs for 20 days. In another experiment, CL (100mg/kg), VA (50 mg/kg) or ORI (50 mg/kg) were administered on Day -1 in the absence of pretreatment with CL and the induction of lethal CDI was compared. For the PK study, the hamsters received a single intravenous (IV) or PO dose (10 to 100 mg/kg). Blood samples and cecal contents were harvested at 8 time points ranging from 15 min to 120h (n=3/time point). ORI levels from plasma samples and cecal contents were determined by LCMS following extraction.
Results: ORI at 10, 50 and 100 mg/kg prolonged survival by 9, 13 and 17 days longer than untreated controls, respectively. On Day 12, ORI at 100 mg/kg exhibited superior efficacy to VA with 100% survival for animals treated with ORI compared to 0% survival with VA. ORI formulated in PEG400 at 100 mg/kg yielded 100% survival at Day 20. CDI was not induced (100% hamsters survived) when ORI alone was administered on Day -1, while 0% survived in CL or VA pretreated groups. After PO administration, the maximal plasma concentration (Cmax) was ≤ 0.12 µg/mL and was between 196-1922 µg/g in cecal content. After IV administration, the concentration in plasma was between 59-279 µg/mL at 15 min and decreased to 0.13-0.52 µg/mL at 48h.
Conclusion: Oritavancin is not bioavailable orally in hamsters and is present at high levels in cecal contents. The high levels found in cecal contents are consistent with the high efficacy of ORI in the hamster model of CDI, in which ORI prolonged survival longer than VA. Moreover, ORI does not induce CDI which suggests that it does not adversely affect the normal gut microflora. These results strongly support further evaluation of ORI for use as a therapeutic agent for CDI in humans.

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IN VITRO ACTIVITY OF FIDAXOMICIN (OPT-80) AND COMPARATOR ANTIMICROBIAL AGENTS AND REA TYPING OF *CLOSTRIDIUM DIFFICILE* RECOVERED DURING A NORTH AMERICAN CLINICAL TRIAL

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Background: With the ever-increasing prevalence of *C. difficile* infection (CDI) and the ongoing search for new therapies, it has been recognized that certain genotypes are associated with the severity of the disease and its recurrence. As reference lab for a recent large clinical trial comparing fidaxomicin with vancomycin, we received fecal specimens collected at pre-therapy (Pre) and at failure (Fr) or recurrence (Rc) from CDI patients across North America. We determined the MICs and REA types and compared the results for strains found in the Pre specimens with those from Fr and Rc.
Methods: Of 631 specimens cultured, we isolated 503 strains of *C. difficile*—434 from Pre, 12 from Fr and 57 from Rc occurring within 4 weeks after treatment. Using the CLSI agar dilution reference method, all isolates were tested for susceptibilities against the two study drugs, FDX and vancomycin (VAN), as well as metronidazole (MTZ), moxifloxacin (MFX) and rifaximin (RFX). Isolates were sent to Dr. Gerding's lab for restriction endonuclease analysis (REA) genotyping.
Results: BI was the most prevalent type, comprising 38.8% of all strains (195/503), with 66.7% (8/12) in the Fr group and 36.8% (21/57) in the Rc group. Other prominent REA types present in the Pre isolates were Y (7.4%), G (7.2%), J (5.6%) and K (3.7%); all except Y were also found in the Rc/Fr group, with J at 11.5% (8/69) and K at 4.3% (3/69). Thirty five % of the Pre strains were non-specific REA types, and 4 were non-typable. MICs (μg/mL) for FDX, VAN and MTZ were ≤0.004 – 0.5, 0.25 – 4, and 0.03 – 4, respectively, for all isolates and had no apparent bearing on cure or Fr/Rc. RFX-R (MIC >256 μg/mL) was present in 8.2% of all strains including 16.4% (32/195) of BI, 3.1% 1/32 of J, and 25% (5/20) of K. The REA Type K had the highest rate of RFX-R at 18.8% (3/16) from the Pre specimens, and 66.7% (2/3) in Rc. MFX-resistance at ≥8μg/mL was found in 47.2% (211/447) of all strains tested, including 88.6% (156/176) of BI, 53% (16/30) of J, and 100% (12/12) of K, but in only 10.3% (3/29) of G and 9.7% (3/31) of Y types. All but 2 RFX-R strains were also resistant to MFX, but not vice versa. Many strains in the Rc/Fr group were the same types as in Pre, and 11 did not have *C. difficile* isolated from Pre with which to compare, but of the remainder 19% (11/58) were new types, including 4 BI, 2 J, 1 G, and 4 non-specified REA.
Conclusions: The BI type was the most prevalent in this study and most often associated with failure and recurrence. Resistance to RFX was higher in Fr/Rc isolates. RFX and MFX-resistance was highest in the BI and K strains.

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GERMINATION OF *CLOSTRIDIUM DIFFICILE* SPORES REPRESENTS A NOVEL STRATEGY TO ENHANCE KILLING BY ULTRAVIOLET RADIATION

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Purpose: Environmental surfaces play an important role in transmission of *Clostridium difficile* and other healthcare-associated pathogens. There is a need for new, rapid disinfection methods that are effective against *Clostridium difficile* spores. We previously demonstrated that ultraviolet radiation administered by the Tru-D™ automated room decontamination device killed *C. difficile* spores, but required ~45 min to decontaminate a hospital room. Because dormant spores become more susceptible to UV radiation upon germination, we hypothesized that triggering germination would enhance killing of *C. difficile* spores on surfaces by the Tru-D device, thereby reducing the time necessary for disinfection of hospital rooms.
Methods: The effect of ultraviolet light on germinating *C. difficile* spores was assessed in the laboratory and on commonly touched surfaces in hospital rooms. Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and dormant *C. difficile* spores were inoculated onto surfaces. The spores were sprayed with a germinant solution consisting of amino acids, minerals and taurocholic acid or sterile water (controls). Quantitative cultures were collected before and after application of ultraviolet reflective doses ranging from 5000-20,000 μWs/cm^2.
Results: MRSA and VRE were significantly reduced at low (~ 10 min) and high (~45 min) reflective doses of radiation; however, dormant *Clostridium difficile* spores required a high dose of radiation to achieve significant reduction. Germination enhanced killing of *C. difficile* spores such that a reflective dose of 10,000 μWs/cm^2 for ~15 minutes was as effective as a 45 minute dose of 20,000 μWs/cm^2. Germinating the spores reduced the frequency of positive cultures on commonly touched surfaces in patient rooms from 78% (44 of 52 sites) to 15% (6 of 27 sites) after a reflective dose of 12,000 μWs/cm^2 for ~20 minutes ($p < 0.001$).
Conclusion: Germination of *C. difficile* spores resulted in enhanced killing by UV radiation on surfaces. This may represent a novel strategy to rapidly eradicate spores from environmental surfaces in hospitals.

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SAFETY AND TOLERABILITY OF AN ORAL SUSPENSION OF VP20621, SPORES OF A NON-TOXIGENIC *C. DIFFICILE* STRAIN; FIRST IN HUMAN ADMINISTRATION TO HEALTHY ADULT SUBJECTS

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Colonization of hamsters with non-toxigenic strains of *Clostridium difficile* is protective against subsequent challenge with toxigenic strains. Recurrence of *C. difficile* infection (CDI) might be prevented in humans by colonizing patients with non-toxigenic *C. difficile* (NTCD) after successful therapy for CDI.

A Phase 1 study was conducted to assess the safety and tolerability of an oral suspension of spores of a non-toxigenic strain of *C. difficile* (VP20621) in healthy adult subjects. Purified spores were produced in a liquid culture medium free of animal-derived components. An oral suspension of 10^4, 10^6, or 10^8 spores or placebo was administered as a single dose to subjects age 18-45 (Group 1, n=15) or ≥60 years of age (Group 2, n=15). In Group 3 (n=12) an oral suspension of 10^8 spores was administered twice daily for 5 days to patients ≥60 years of age. All subjects were followed through Day 28. *C. difficile* stool cultures were performed at various time points; all *C. difficile* isolates were tested for the production of toxin by enzyme immunoassay.

Blinded results show that study drug was well tolerated. No serious or severe adverse events (AEs) were observed and no subjects discontinued study drug. The most common AEs were headache (n=3), fatigue (n=2), and burning sensation of the tongue (n=2). No subjects reported AEs of diarrhea or change in stool form or frequency. Groups 1 and 2: no *C. difficile* was cultured from stool samples. Group 3: *C. difficile* was detected in stool cultures from 7/12 subjects by day 3, in 6/12 on day 7, and 0/12 on days 14 & 28. No toxin was detected in isolate supernatants. Further characterization of isolates is ongoing.

This Phase 1 study showed VP20621 to be well tolerated in younger and older volunteer subjects. The finding of NTCD in stool after multiple-dose administration in this study likely represents "pass-through" detection. Evaluation of VP20621 administered after pre-treatment with oral antibiotic therapy is planned.

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SPORICIDE ACTIVITY OF DISINFECTANTS USED AT HOSPITAL CLEANING ROUTINE AGAINST SPORULATED FORMS OF *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile is an obligate anaerobe Gram-positive bacterium capable of forming endospores. It is considered the main agent of pseudomembranous colitis and antibiotic-associated diarrhea cases. Patients with *C. difficile* associated diarrhea (CDAD) excrete a great number of vegetative cells and *C. difficile* endospores in stool. This can lead to the contamination of the environment, which is one of the most important reservoirs and transmission area of *C. difficile* spores. Due to its greater resistance to adverse conditions, the spores can persist in the nosocomial environment for much longer periods than its vegetative form. Since most of the cases of infection by *C. difficile* occur after hospitalization, it is necessary to determine agents that could effectively eliminate those spores from the environment. Therefore, the aim of this study is to evaluate the sporicide activity of disinfectants commonly used at hospital cleaning routine against *C. difficile* spores, such as Virkon (Potassium Persulphate), Hypochlorite and Chlorhexidine. A *C. difficile* strain (Amb3) isolated from a lavatory sink from a hospital in Rio de Janeiro, Brazil was used as a test strain. The spores were obtained after incubating the strain on BHI agar anaerobically during 5-7 days to induce sporulation and heat shock was used to inactivate vegetative cells. The disinfectant Virkon was incubated with the spore suspension and neutralized with sodium thiosulfate at increasing time points. Different dilutions of the suspension were spread on BHI agar containing sodium taurocholate 0.1%, for CFU count. It was observed a time dependant reduction in bacterial survival, suggesting a possible sporicide activity of Virkon. We believe that our work will help the Brazilian hospitals to make improvements in their disinfection protocols and help prevent the occurrence of CDAD in hospitalized patients.

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CLOSTRIDIUM DIFFICILE: TREATMENT OPTIONS

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *CLOSTRIDIUM DIFFICILE* IN A TURKISH UNIVERSITY HOSPITAL

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Clostridium difficile-associated diarrhea is common in hospitals and usually treated with metronidazole or vancomycin. However, resistance to metronidazole and intermediate resistance to vancomycin were recently reported. This emphasizes the need for periodic monitoring of any emergence of drug resistance in *Clostridium difficile*.

The aim of the present study was to investigate its susceptibility to metronidazole, vancomycin, meropenem, clindamycin and ampicillin. Toxigenic *Clostridium difficile* isolates (n:50) collected at Marmara University Hospital between April 2008 and January 2010 were studied. The MICs of the isolates for five antibiotics were determined using the agar dilution protocol in the CLSI guidelines (M11-A7). The antibiotics and concentrations used were as follows: 32–0.125 mg /L for metronidazole, 16–0.125 mg /L for vancomycin, 32–0.125 mg /L for meropenem 256–0.125 mg /L for clindamycin, and 16–0.125 mg /L for ampicillin. Breakpoints of susceptibility for each drug were chosen at the levels listed by the CLSI, intermediate strains evaluated as resistant ones. All isolates were sensitive to metronidazole, vancomycin and meropenem with a narrow range of MICs. The MIC50/MIC90 values (mg/L) of strains were 0.125/0.25 against metronidazole, 0.5/1against vancomycin and 1/2 against meropenem. Resistance to clindamycin was seen in 31 isolates (62 %) with MIC50 and MIC90 values 4 and 16mg/L, respectively. Similar high resistance rate to ampicillin (68 %) was also found. Their MIC50 values (1mg/L) were high and the same as their MIC90 values. For the present, it looks like there was no resistance risk for metronidazole and vancomycin, the two agents commonly used to treat *Clostridium difficile*-associated diarrhea. Our findings determine the current antibiotic susceptibility patterns of the *Clostridium difficile* isolates in our region. These results also give a clue for Turkey, where clinical laboratories do not routinely perform culture and susceptibility testing of the organism.

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CLOSTRIDIUM DIFFICILE: TREATMENT OPTIONS

TREATMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION FOR MORE THAN 10 DAYS DOES NOT REDUCE DEATHS OR RECURRENCE

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Statement of Purpose: The exact reasons for high rates of recurrence and mortality in *Clostridium difficile* Infection (CDI) remain unclear; this study was designed to assess the impact of treatment duration on outcomes.
Methods: A prospective, observational study was performed from 2005-2006. Patients were initially identified by a positive *C. difficile* (CD) toxin assay. All positive samples were sent for isolation of the CD organism. Patients were included in the analysis if a CD isolate was recovered. Clinical and demographic data were collected. Outcomes were measured by cure, recurrence and 30 day all-cause death. Recurrence was defined as symptoms plus a positive CD toxin assay within 8 weeks of the initial episode.
Results: There were 118 patients with CD isolates recovered from their stool sample. The mean age was 67.9 years, and 59.3% were female. The 30-day all cause mortality was 24.6% (29) and 98% (105/107) of the patients were initially treated with metronidazole. The mean treatment duration was 11.67 days. In assessing treatment duration, excluding all-cause deaths within the initial 5 days of diagnosis, the mortality rate for those treated ≤10 days was 13.9% (5/36) vs. 16% (8/50) for those treated >10 days ($p=0.79$, χ^2). In addition, the mean treatment duration for those who died > 5 days after diagnosis was similar to survivors, 11.5 days vs. 12.9 days ($p=0.5$, Student's t-test). When we included all deaths in the analysis, the mean treatment duration in those who died vs. survivors, was 7.7 days vs. 12.9 days ($p=0.003$, Student's t-test). The recurrence rate among survivors was 23.6% (21/89). Recurrence occurred in 22.6% (7/31) of patients treated for ≤10 days vs. 28.6% (12/42) for those treated >10 days ($p=0.56$, χ^2).
Conclusions: While patients with CDI who died within 30 days of diagnosis appeared to receive shorter courses of treatment, there were no differences in mortality rates or treatment duration if patients who died within 5 days of diagnosis were excluded. Recurrence rates were also not affected by treatment for more or less than 10 days.