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Philadelphia, PA USA • July 7-10, 2010

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

VARIATIONS IN TCDB AND THE HYPERVIRULENCE OF *CLOSTRIDIUM DIFFICILE*—NAP1

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Clostridium difficile is the leading cause of hospital-acquired diarrhea, and there has been a concerning increase in the morbidity and mortality of this illness. This increase in disease severity is due in part to the emergence of variable hypervirulent strains, including *C. difficile* NAP1. Our current study seeks to understand why this newly emerging strain of *C. difficile* is more virulent than the historical strain. A major virulence factor of *C. difficile* is the cytotoxin TcdB, which is responsible for part of the pathology of the intestines as well as systemic effects. Interestingly, sequence analysis has shown that TcdB from the NAP1 strain has significant variations from TcdB from the historical strain. In this study we demonstrate that TcdB-NAP1 exhibits variable activity and tropism by using cytotoxic analyses and a zebrafish model of intoxication. We have now shown that this variation in activity is not due to differences in the intracellular activity of TcdB, leading us to focus on defining interaction and internalization into host cells. We utilized cell culture intoxication experiments that have provided interesting information on the nature of the cell association and mechanism of cell entry. Two striking differences were observed between TcdB-NAP1 and TcdB of historical strains. First, antibodies raised against the putative receptor binding domain region of TcdB are not cross neutralizing and second, the time course of cell entry along with the requirement for endosomal acidification is reduced dramatically for TcdB-NAP1 in some cell types. This suggests TcdB may use a previously undefined mechanism of cellular intoxication, and differences in this process could account for the variation between the cytotoxicity of the two forms of this toxin. Collectively, these results suggest sequence variation of TcdB may play a role in the hypervirulence of *Clostridium difficile* NAP1.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

THE GASTROINTESTINAL MICROBIOTA AND *CLOSTRIDIUM DIFFICILE*

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Antibiotic-associated diarrhea due to infection with *Clostridium difficile* is thought to arise in this setting out alterations in the indigenous gut microbiota. Until recently, the study of complex microbial communities was limited by the culture-based methodologies that only captured a subset of the diversity present. The advent of molecular methods for the study of microbial consortia has allowed us to greatly increase our understanding of the structure, function, and dynamics of the gut microbiota. We have recently applied culture-independent molecular methods, largely based on retrieval of 16S rRNA-encoding gene sequences, to investigate the role of the indigenous microbiota in *C. difficile* infection (CDI). Through studies of human patients with CDI we have demonstrated that recurrent disease is associated with an overall decrease in the diversity of the fecal microbiota. We have leveraged a recently-described murine model of CDI to conduct more detailed studies of the role of the microbiota in *C. difficile* infection. Data from this model system indicates that a subset of the indigenous microbiota is important in mediating colonization resistance against *C. difficile* and possibly in modulating the effects of *C. difficile* toxin. Studies of the microbial ecology of the indigenous gut microbiota in both human patients and murine models will increase our understanding of the pathogenesis of CDI and may lead to novel methods for prevention and treatment of this reemerging infectious disease.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

THE ROLE OF THE TOXINS TCDA AND TCDB IN *CLOSTRIDIUM DIFFICILE* INFECTION

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The two large toxins, TcdA and TcdB, are thought to be the main virulence factors in *Clostridium difficile* infection (CDI). Up to date it has been difficult to study their relative roles in disease due to lack of genetic tools in Clostridia. The two toxins are members of the clostridial glucosylating toxin family, which comprises some of the largest known bacterial toxins. They enter the cytosol of target cells via receptor-mediated endocytosis and then act by transferring glucose onto Rho GTPases, inactivating them. This causes the disruption of various signalling pathways, leading amongst others to changes in the organization of the actin cytoskeleton and ultimately to cell death. Historically, toxin A was believed to have a dominant role in disease. However with the emergence of A^-B^+ strains it was recognised that toxin B alone can also cause disease. A recent study, in which unstable, single crossover mutants were generated, indicated that toxin A alone cannot cause disease in hamsters[1]. This leaves a paradox over the individual importance of toxin A and toxin B in CDI. Making use of newly available genetic tools for Clostridia, we constructed stable, isogenic single and for the first time double mutants of *tcdA* and *tcdB.* The mutants were generated using the ClosTron mutagenesis system[2]. They were characterised, analysed and tested *in vitro* (including cytotoxicity assays) and *in vivo* using the hamster infection model. Each toxin alone displayed cytotoxic effects, whereas no toxicity was measured in the double mutant (A^-B^-). Furthermore our findings re-establish the importance of both toxin A and toxin B and show that each toxin alone can cause fatal disease in the hamster infection model.

References
[1]Lyras, D. *et al*. Toxin B is essential for virulence of *Clostridium difficile*. Nature 458, 1176-9 (2009).
[2]Heap, J. T. *et al*. The ClosTron: Mutagenesis in *Clostridium* refined and streamlined. J Microbiol Methods 80, 49-55 (2010).

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

THE ROLE OF TCDC IN THE VIRULENCE OF *CLOSTRIDIUM DIFFICILE* NAP1/027 EPIDEMIC STRAINS

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Clostridium difficile is an emerging pathogen, which in recent years has been recognised as an increasingly important cause of nosocomial disease. *C. difficile* infections can range from relatively mild diarrhea to pseudomembranous colitis, toxic megacolon, shock and death. More recently, epidemic NAP1/027 isolates have been associated with more severe disease and higher rates of mortality. These strains are reported to produce significantly more toxin than other clinical isolates and this is thought to be due to the presence of a nonsense mutation within the *tcdC* gene, which encodes a negative regulator of toxin production. To more effectively study NAP1/027 epidemic strains at the molecular level we developed a new and more efficient plasmid transfer system that facilitated the complementation of a NAP1/027 epidemic isolate with an intact copy of *tcdC*. Subsequent analysis by Western blotting, cell cytotoxicity assays and qRT-PCR showed that *in trans* provision of *tcdC* results in significant repression of toxin production in this strain. These data represent the first *in vivo* demonstration in *C. difficile* that *tcdC* negatively regulates toxin production and support the hypothesis that *tcdC* mutations present in NAP1/027 strains can lead to increased levels of toxin production.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

REQUIREMENT FOR GERMINANT RECOGNITION BY *C. DIFFICILE* SPORES

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Germination of *Clostridium difficile* spores is the first required step in establishing *C. difficile* associated disease (CDAD). Recently, taurocholate and glycine were shown to be required for spore germination *in vitro*. In the present study, we tested a series of glycine and taurocholate analogs for their ability to induce or to inhibit *C. difficile* spore germination. These assays allowed to determinate taurocholate and amino acid functional groups required to bind and/or activate putative germination receptors. Testing of glycine analogs revealed that both the carboxy and amino groups are important epitopes for recognition and that the glycine-binding site is flexible enough to accommodate compounds with more separated carboxy and amino groups.

The *C. difficile* germination machinery also recognizes other amino acid side chains. In general, linear alkyl side chains are better activators of spore germination than their branched analogs. However, L-phenylalanine is also a good germinant. We expect that since branched amino acids are excluded, L-phenylalanine's aromatic side chain should not be able to fit in the same binding site as alkyl amino acids. Thus, aromatic amino acids are probably recognized by a separate binding site. L-arginine is also a strong germinant for *C. difficile* spores. In contrast, L-lysine, which also has a linear, positively charged, side chain, does not affect spore germination. Thus, it seems that there is also a separate, arginine specific binding site.

Testing of taurocholate analogs revealed that *C. difficile* spores are able to detect shorter, but not longer, amino sulfonic acid side chains. Furthermore, the sulfonic acid group can be partially substituted with other acidic groups. Finally, the 12-hydroxyl group is necessary, but not sufficient to activate spore germination. In contrast, the 6- and 7-hydroxyl groups are required for binding but not activation of *C. difficile* spore germination. In conclusion, *C. difficile* spores interact with both amino acids and taurocholate thorough multiple interactions that are required to recognize the germinant and/or activate the germination machinery.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

KINETIC EVIDENCE FOR THE PRESENCE OF PUTATIVE GERMINATION RECEPTORS IN *CLOSTRIDIUM DIFFICILE* SPORES

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Clostridium difficile is a spore-forming bacterium that causes *Clostridium difficile* associated disease (CDAD). Intestinal microflora keeps *C. difficile* in the spore state and resists colonization. Following antimicrobial treatment, the bacterial microflora is disrupted and *C. difficile* spores germinate in the intestines. The resulting vegetative cells fill empty niches left by the depleted microbial community and establish infection. Thus, germination of *C. difficile* spore is the first required step in CDAD establishment. Interestingly, the *C. difficile* genome encodes every spore-specific protein necessary for germination, except for germination receptors. Even though *C. difficile* germination receptors have not been identified, taurocholate (a bile salt) and glycine (an amino acid) were shown to be required for spore germination. Furthermore, chenodeoxycholate, another bile salt, can inhibit *C. difficile* spore germination. In the present study, we examined *C. difficile* spore germination kinetics to determine whether taurocholate acts as a specific germinant that activates unknown germination receptors or acts non-specifically by disrupting spore membranes. Kinetic analysis of *C. difficile* spore germination suggested the presence of distinct receptors for taurocholate and glycine. Furthermore, taurocholate, glycine, and chenodeoxycholate seem to bind to *C. difficile* spores through a complex mechanism, where both receptor homo- and heterocomplexes are formed. The kinetic data also points to an ordered sequential progression of binding where taurocholate must be recognized first before detection of glycine can take place. Finally, comparing calculated kinetic parameters with intestinal concentrations of the two germinants suggested a mechanism for the preferential germination of *C. difficile* spores in antibiotic treated individuals.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

INTESTINAL EPITHELIAL MITOGEN ACTIVATED PROTEIN KINASE ACTIVATED PROTEIN KINASE 2 (P-MK2) IS ELEVATED BY *CLOSTRIDIUM DIFFICILE* TOXIN

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Purpose: To determine if P-MK2, a stabilizer of inflammatory and apoptotic mediators in human inflammation, is elevated by *C. difficile* toxin (Tcd). **Methods**: A capture enzyme immunoassay (EIA) was developed to quantify P-MK2 concentrations in cultured T-84 intestinal tissue-cultured cells stimulated with standardized toxigenic culture filtrate dilutions. Additionally, P-MK2 levels were assayed in a pilot study using 10 mg stool lysates each, from fifteen retrospective, cross-sectional, banked samples of hospitalized, pediatric patients with and without toxigenic *C. difficile*. All stools were initially tested by *C. difficile* TcdA/B EIA, and had subsequent adjunctive culture-*TcdB* PCR for a 144-bp gene fragment at the time of P-MK2 EIA.
Results: P-MK2 EIA demonstrated linearity from 1 to 500 pg/µl of pure P-MK2 protein standard. Tissue cultured-cells exhibited approximately 50% cytopathic effect at about 6 hr post-culture filtrate stimulation with 27.3 mg/ml and approximately 20% with 2.73 mg/ml of culture filtrate protein, but not in antitoxin-neutralized or unstimulated tissue-cultured cells. P-MK-2 levels were 75, 37, 0, and 0 pg/µl, for 27.3 and 2.73 mg/ml of culture filtrate protein, antitoxin-neutralized, and unstimulated tissue-cultured cells, respectively, ($p=0.0087$, unpaired t-test). In human stools, we saw a trend towards P-MK2 elevation if toxigenic *C. difficile* was present (n=8, 803 ± 345) compared with the absence of toxigenic *C. difficile* (n=7, 200 ± 44), ($p=0.156$, Mann-Whitney U).
Conclusions: Correlation of P-MK2 level with dose-response and neutralization of cytotoxin in human intestinal tissue-cultured cells, and P-MK2 elevation in humans with toxigenic *C. difficile*, suggest that P-MK2 may play a role in *C. difficile* infection. *In vitro* studies with purified TcdB and larger human studies are needed to further clarify the role of P-MK2 in *C. difficile* infection.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

CLOSTRIDIUM DIFFICILE BINARY TOXIN (CDT) IN ANTIBIOTIC ASSOCIATED DIARRHEA

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We used an EIA, calibrated with purified recombinant antigen, to quantify *cdtB* which acts as the binding component of *Clostridium difficile* binary toxin (CDT). This work was done with feces from 80 confirmed human cases of *C. difficile* diarrhea and culture fluids of isolates recovered from 53 of the same fecal samples. By using PCR, we ribotyped isolates and probed each for the three genes (*cdtR*, *cdtA*, *cdtB*) that make up the CDT locus. CDT is best known as a toxin associated, though not exclusively so, with the ribotype 027 isolates that are central to the recent worldwide rise in the incidence *C. difficile* diarrhea. We found $CDT^{(+)}$ isolates in 50/80 (63%) fecal samples; *cdtB* was present in only 38% of that 50. In contrast, 90% of all $CDT^{(+)}$ isolates we recovered made *cdtB* protein in broth culture. Ribotype 027 isolates were 86% of all $CDT^{(+)}$ isolates. Six other ribotypes accounted for the 7 non-027, $CDT^{(+)}$ isolates; 6 of the 7 made cdtB *in vitro*. 25/80 (31%) isolates carried the ghost CDT locus, i.e. an intact *cdtR* but only fragments of *cdtA* and *cdtB*. 5/80 (6%) lacked all or any part of the CDT locus. No CDT^{-} isolate was recovered from an EIA negative stool sample. Levels of *cdtB* in the fecal samples were on average nearly twenty-fold greater than the corresponding isolates achieved *in vitro*. We saw, however, no direct relationship between toxin concentrations in feces and *in vitro* cultures. Over half of the EIA positive samples contained >100 ng *cdtB*/mL, a level matching that reached in stool during other closely related clostridial binary toxin diarrheas. This information suggests that when there is 100 ng/mL or more of *cdtB* present, the level of CDT may be sufficient to cause diarrheic symptoms, possibly complementing the known enteric activities of Toxins A and B.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

SPORULATION EFFICIENCY OF *CLOSTRIDIUM DIFFICILE* IN FOUR PRE-REDUCED, ANAEROBICALLY-STERILIZED BROTH FORMULATIONS

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The utility of a commercially-prepared broth formulation for the reliable and reproducible production of *Clostridium difficile* spores facilitates a variety of research applications. To determine which formulation is most suitable for spore production, four Pre-Reduced, Anaerobically-Sterilized (PRAS) broth formulations, including Brain Heart Infusion Broth (BHI), Peptone Yeast Extract Broth (PY), Duncan and Strong Sporulation Broth (DS) and Wilson's Sporulation Broth (SB), were compared for their ability to allow *Clostridium difficile* ATCC 700057 to form spores. The strain was grown in triplicate, in each of the four media, for each time point. Each broth was subcultured —at 24, 48, and 72 hours—and serially diluted to determine the total viable count. The replicates were then heat activated and the vegetative forms killed, by heating at 70°C, for 10 minutes. Serial dilutions were then performed and subsequently plated on two PRAS agars: Brucella Blood Agar (BRU) and Brain Heart Infusion agar with Horse Blood and Taurocholate (BHI-HT). Colony-forming units were then counted and compared for each broth formulation and the ability of BRU and BHI-HT to recover both vegetative and spore forms of *C. difficile*. Wilson's Sporulation Broth (SB) consistently produced the greatest significant quantity of spores recovered on either agar ($P < 0.005$). Brucella Blood Agar (BRU) recovered a higher quantity of total viable cells and spores from the BHI and PY broth formulations as compared to the BHI-HT; however, the BHI-HT agar recovered more total viable cells and spores from the SB and DS broth formulations ($P < 0.005$). Based on this study, it appears that the SB formulation is capable of reproducibly generating the highest number of viable spores from *Clostridium difficile* strain 700057, and that the BHI-HT agar formulation is valuable in optimizing the recovery of total viable cells and heat-treated spores.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

GLYCINE STIMULATES AND HYDROXYPROLINE SUPPRESSES TOXIN PRODUCTION IN *C. DIFFICILE*

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Clostridium difficile associated disease is an increasing problem within the healthcare setting. Pathogenicity is mediated by two toxins, TcdA and TcdB. Their cytotoxicity is due to glucosylation of Rho GTPases resulting in depolymerization of the actin cytoskeleton, mucosal damage and inflammation. Previous studies have shown that toxin production can be affected by composition of the growth medium, yet regulation of toxin gene expression in response to environmental stimuli is not well understood. Our previous work described the presence of Stickland reactions in *C. difficile*. Stickland fermentation is the coupled oxidation (donor) and reduction (acceptor) of amino acid pairs. In this study we examine the impact of the addition of Stickland acceptor amino acids on growth kinetics and yield, protein synthesis, toxin production and gene expression. *C. difficile* was cultivated in rich medium with the addition of added Stickland acceptor amino acids (Stickland acceptors; glycine, L-proline, and L-hydroxyproline). Optical density and protein concentrations were measured throughout the growth period. Extracellular toxins were analyzed by SDS-PAGE and western blot. mRNA was isolated from cultures and real time RT-PCR was utilized to examine gene expression. Addition of Stickland acceptors to the growth media significantly alters toxin expression. Glycine, in particular, significantly increases the amounts of extracellular protein and toxin. In contrast, addition of L-hydroxyproline leads to near complete suppression of toxin production. A recently described regulator of toxin production, CodY, could play a role in this regulation. However we found that a *codY* mutant still produced more toxin (Toxin A as measured by immunoblot) in response to the addition of glycine. The results indicate that the effect of Stickland reactions on toxin production could be independent of CodY. Moreover this suggests that another layer of regulation exists that allows the cell to respond to the amino acid composition for regulation of toxin production, and the data suggest there is a link between Stickland reactions and toxin regulation.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

INHIBITION OF *CLOSTRIDIUM DIFFICILE* SPORE GERMINATION BY CHEMICAL ANALOGS OF CHENODEOXYCHOLATE

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Spore formation by *Clostridium difficile* is a significant obstacle to preventing the spread of *C. difficile*-infection. Spores are resistant to heat, radiation, chemicals and antibiotics, making a contaminated environment difficult to clean. In the host, spores must germinate and grow out as vegetative cells and this process requires bile salts. Primary bile salts produced by the liver are composed mainly of cholate and chenodeoxycholate conjugated with either taurine or glycine We have shown that some cholate derivatives can act with glycine to induce the germination of *C. difficile* spores. Chenodeoxycholate was unable to stimulate the germination of *C. difficile* spores but was able to inhibit the growth of vegetative bacteria. In fact, we have shown that chenodeoxycholate can also competitively inhibit spore germination induced by cholate or taurocholate. The colonic flora metabolizes chenodeoxycholate to lithocholate by 7α-dehydroxylation. Here we tested analogs of chenodeoxycholate that are predicted to resist 7α-dehyoxylation by the normal colonic flora, for their ability to inhibit *C. difficile* spore germination. By spectrophotometrically measuring the initiation of germination, we found that *C. difficile* spores germinate with apparent Michaelis-Menten kinetics. Using kinetic analysis of *C. difficile* spore germination we assigned apparent inhibitor constants (K_i) to chenodeoxycholate and chenodeoxycholate analogs, allowing a more quantitative measure of inhibition of germination. We found that some compounds are more potent inhibitors of *C. difficile* spore germination. Future work will determine if these analogs resist 7α-dehydroxylation by a member of the colonic flora and their effect as inhibitors of *C. difficile* disease.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

UPDATE ON NORTH AMERICAN PULSED FIELD GEL ELECTROPHORESIS (NAP) TYPES OF *CLOSTRIDIUM DIFFICILE* IN THE UNITED STATES

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Purpose: To describe the characteristics and frequencies of recently-named *Clostridium difficile* North American pulsed field gel electrophoresis (PFGE) types, NAP7 through NAP12, among isolates in the Centers for Disease Control and Prevention collection.
Methods and Results: Since 2003, a total of 1,741 *C. difficile* isolates from the CDC strain collection have been analyzed by PFGE. These represent human, animal, and retail meat isolates in the United States and Canada, primarily from studies and outbreak response activities. Six North American PFGE (NAP) types have been previously described on the basis of frequency, overall geographic and temporal distribution, and the consistency of molecular characteristics. Consultation between the CDC and the Public Health Agency of Canada (PHAC) has resulted in the assignment of six new types, NAP7–NAP12. Isolates are assigned to one of these types on the basis of *Sma*I PFGE, with an 80% similarity threshold to the prototypical PFGE pattern (Dice/UPGMA). As of December 2009, strain types NAP1–NAP6 represented 50.1% (n=872) of the isolates in the CDC database, with NAP1 representing the majority of all isolates (n=585; 33.6%). NAP7– NAP12 strains comprise 16.9% (n=294) of isolates in our collection. NAP7 and NAP8 were assigned to toxinotype V, binary toxin positive isolates with a 39bp deletion in *tcdC*. These strains are most common in food-producing animals but also cause human disease. NAP7 and NAP8 have between 5 and 7 measurable bands by *Sma*I PFGE and encompass PCR-Ribotypes 078 and 126, respectively. NAP7 represents 10.9% (n=190) and NAP8 thru NAP12 represent less than 2% each of total isolates in the CDC collection. NAP9 isolates are toxinotype VIII, PCR-Ribotype 017, and binary toxin positive with no deletion in *tcdC*. The remaining three novel NAP type patterns, NAP10, NAP11, and NAP12 are all toxinotype 0, binary toxin negative; only NAP12 isolates have a characteristic 18bp deletion in *tcdC*. NAP10, NAP11 and NAP12 generally correlate with PCR-Ribotype 070, PCR-Ribotype 106, and PCR-Ribotype 015, respectively.
Conclusion: NAP7 through NAP12 are recent additions to the *C. difficile* strain typing nomenclature, and include several important and emerging strain types. CDC and PHAC are continuing surveillance activities to monitor emerging *C. difficile* strains, and will continue to identify candidate strains for NAP designation based on frequency of isolation and geographic distribution.

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CLOSTRIDIUM DIFFICILE: PATHOGENESIS

MICROBIOTA STRUCTURE, *CLOSTRIDIUM DIFFICILE* GENETIC DIVERSITY, AND *CLOSTRIDIUM DIFFICILE* INFECTION

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The purpose of this study is to examine if the human gastrointestinal (GI) tract microbiota influences the outcome of the host-microbe interaction between humans and *Clostridium difficile*. For approximately 5-15% of healthy people, *C. difficile* is a minor component of the GI tract microbiota. The main risk factor of symptomatic *C. difficile* infection (CDI) is antibiotic therapy, which also has a profound impact on the abundance and distribution of bacteria in the body. A number of details concerning the initiation of *C. difficile* virulence remain enigmatic. Some of them include: Are the same genotypes of *C. difficile* carried with equal frequency among young (<65) and old (65 and older) patient populations? Is a specific GI tract bacterial community associated with asymptomatic carriage of *C. difficile*? How often are genotypes that cause clinical disease (CDI) carried asymptomatically? To begin to answer these questions, we are characterizing the GI tract microbiota and *C. difficile* isolates from patients with formed stools (no clinical symptoms of CDI) and patients with CDI. We have recently initiated collection of 40 stool samples per week from patients with suspected CDI (stools sent to the clinical lab for CDI testing) and from asymptomatic patients (formed stools from stools sent to the clinical lab for fecal occult blood testing). As part of our IRB-approved collection protocol, we obtain patient information so that comparisons between patient groups can be made. To date, we have collected 250 stool samples (70 with CDI, 70 without CDI, and 110 from asymptomatic patients) and cultured 80 *C. difficile* strains using selective media (TCCFA agar plates). For each isolate, we have screened for the presence of toxin genes, generated ribotype profiles, and performed multilocus variable-number tandem repeat analysis (MLVA) on isolates representing the same ribotype. We have also initiated a non-culture based characterization of the structure of the GI tract microbiota from CDI and asymptomatic patients. These data will be used to determine if *C. difficile* carriage is associated with the presence/absence of certain bacterial constituents of the patient's GI tract microbiota.

Anaerobe 2010
The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

CLOSTRIDIUM DIFFICILE: PATHOGENESIS

CHARACTERISATION OF *CLOSTRIDIUM DIFFICILE* RIBOTYPE 027 STRAINS IN SCOTLAND

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Clostridium difficile has been recognised as the cause of antibiotic-associated diarrhoea since 1978. Several different PCR ribotypes have been identified, but one in particular has caused extensive havoc in the medical community. This is the hypervirulent strain we know as PCR ribotype 027 (NAP1/BI). It has been associated with several outbreaks and disease with greater severity, higher mortality and more recurrences. Though first identified in the UK in 2004, the first case in Scotland was seen only in late 2006. The frequency of ribotype 027 in Scotland has increased from about 3% in early 2008 to about 13% at the end of 2009. The hypervirulence of this strain has been attributed several factors: excessive toxin production, which might be caused by mutations in *tcdC* (the negative regulator of toxin production), the presence of the binary toxin, the use of fluoroquinolones and a corresponding mutation in the *gyrA* gene. Seven ribotype 027 isolates from different parts of Scotland were collected and their phenotypic and genotypic characteristics were studied. The isolates were ribotyped and toxinotyped and confirmed to be ribotype 027, toxinontype III. They all carry the genes for the binary toxins CDTa and CDTb. Though there was no significant difference in the growth of the ribotype 027 strains compared to the reference strain 630 at 24 h, they produced significantly higher amounts of toxin (A and B). Sequence analysis of the *tcdC* gene revealed that they all carried the Δ117 deletion as well as the 18bp deletion. Further, all the 027 isolates were moxifloxacin resistant and have the Thr82Ile mutation in the *gyrA* gene. However, no mutations were found in the *gyrB* gene of any of the isolates. Further investigations into the sporulation and antibiotic resistance are being carried out in order to better understand this ribotype and the reasons for its increasing prevalence in the population.