INV1

MILESTONES IN *CLOSTRIDIUM DIFFICILE* RESEARCH

Dale N. Gerding, MD

Pseudomembranous colitis (PMC) was first described in 1893 and *Clostridium difficile* was discovered in 1935. *C. difficile* was not identified as etiologic of PMC until 1978 when it was found to be the cause of "clindamycin colitis" which was first described in 1974, linking specific antibiotic exposure and subsequent PMC. The need for both prior antibiotic exposure and exposure to *C. difficile* was demonstrated in the hamster model (1980). Diagnosis was established by cell cytotoxin testing of stool in 1978, by culture of stool on selective media in 1979, and by enzyme immunoassay for toxin A in 1983. Hospital environmental contamination was identified in 1979 and hand contamination of healthcare worker hands and home environments in 1981. Vancomycin was quickly identified as a treatment agent through use of the hamster animal model (1977). Human trials also identified metronidazole as effective treatment (1983). Serum antibody to toxin A was found in 64% and to toxin B in 66% of adults (1983). Recovery from CDI and absence of relapse was found to correlate with serum antitoxin B antibody and neutralization (1985). Early purified toxin experiments suggested toxin A was more important in pathogenesis than toxin B based on oral administration of the toxins to hamsters (1985). A 1986 case-control study in hospitalized patients identified clindamycin and receipt of multiple treatment antibiotics as risk factors for nosocomial (87% of all cases) *C. difficile* associated disease (CDAD,). Hospital acquisition of *C. difficile* occurred in 21% of all patients, but 63% were asymptomatic (1989). In 1990 the toxin A and B genes were cloned and sequenced and in 1995 the toxin mechanism of action; glycosylation of small GTP-binding proteins (Rho subfamily) involved in cell cytoskeleton organization was described. In 1998 a system of toxinotyping based on genetic variations in the pathogenicity locus was described. Prevention of CDAD was shown by restriction of clindamycin in 1994, by cephalosporin control in 1997, and by use of sporicidal environmental bleaching in 2000. In the 21st century the term CDAD was converted to *C. difficile* infection (CDI), and a rise in anti-toxin A serum antibodies was associated with protection from symptomatic CDI (2000) and prevention of CDI recurrence (2001). The major research developments of the 21st century have been the reports of the NAP1/BI/027 epidemics (2005) and their rapid spread (2013), the description of a mouse model for CDI (2008), waning efficacy of metronidazole treatment (2007,2014), fidaxomicin, the first new treatment antibiotic for CDI (2011), potential for recurrence prevention with monoclonal antibodies (2010), commercial development of PCR diagnostic testing, development of toxin mutants of *C. difficile* to test pathogenesis (2009, 2010) and rising interest in fecal microbiome therapy for recurrent CDI (2013).

INV2

THE SPECTRUM OF *CLOSTRIDIUM DIFFICILE INFECTION (CDI)* IN A GENERAL HOSPITAL. CASES AND SITUATIONS FROM THE REAL LIFE.

Emilio Bouza

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The real spectrum of CDI requires a policy of systematic search for toxigenic *C. difficile* in all diarrhoeic stools received from hospitalized and non-hospitalized patients. Overall, in a large hospital area serving a population of approximately 715.000 inhabitants, we increased 12.7% the CDI episodes detected with this policy. A second enteric pathogen was present in 2.5% of the patients with CDI. Determination of the origin of the episodes requires a direct interview with the patients. In our institution, 74.5% were Health-Care Related, 18,6% had a Community Onset and in 6.9% the origin cound not be clearly determined. Community-Acquired cases can go more easely undetected because they occur in a younger population with less "classic" predisposinng conditions.

Overall, 83.8% of our episodes had a moderate presentation, 14,7% were severe and 1.5% were severe-complicated. Recurrences occurred in 16.2% of the whole population and non-response in 4%. (Overall Poor Outcome 20.2%).

Prediction of a PO (either due to evolution or recurrence) at the beginning of the first episode was particularly difficult on the basis of clinical data but the prediction can be improved including in the prediction models data on the toxigenic load of the patients measured either by colony counts, toxin levels or cycles of amplificación in molecular tests in stools.

The problema of CDI in different populations will be discussed including CDI in Intensive Care Units, Pediatric patients, Solid Organ Transplant Patients and the HIV infected populations. In our own data, the episodes of CDI occurring in ICU's represent less tan 5% of the episodes and those not present on admission have severity and evolution comparable to the episodes occurring outside ICU's. Reports of a high increase of episodes in the pediatric population my represent more a better policy of clinical awareness and microbiological searching than a real increase of this condition.

Regarding management we will discuss the approach to patients presenting in outbreaks, particularly those of ribotype 027, and the potential need for more prolonged treatment with oral vancomycin.

INV3

THE "ONE HEALTH" PARADIGM AND *C. difficile*

Thomas V Riley

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The One Health concept is a worldwide strategy for interdisciplinary collaboration and communication in all aspects of healthcare for humans, animals and the environment. In recent years, 70% of emerging or re-emerging infections have been vector-borne or zoonoses - animal diseases that are transmissible to humans. Most attention has focused on viral infections because of highly publicised outbreaks; SARS, avian influenza, "swine-flu" and ebola. However, disease associated with *Clostridium difficile* infection (CDI) has killed more people world-wide in the last 15 years than all these viral infections combined! CDI should always have been considered a zoonosis, either direct or indirect. In some definitions of zoonoses, non-human animal hosts play an essential role in maintaining the infection in nature and humans are only incidental hosts. In CDI, all animals (human and non-human) are likely hosts; the wide variety of animals from which *C. difficile* has already been isolated suggests this. What then is the natural history of CDI? *C. difficile* is ubiquitous in the environment. It would appear that *C. difficile* colonises the gastrointestinal tracts of all animals during the neonatal period, multiplies and is excreted, but cannot/does not compete well when other bacterial species start to colonise. The exact timing of this change is unclear but it is probably linked to changes in diet in human babies, i.e. weaning. Adult humans treated with antibiotics fool *C. difficile* into thinking it is colonising a neonatal gut. When those antibiotics were cephalosporins in the 1980s and 90s, antibiotics to which *C. difficile* is intrinsically resistant, there was an expansion of CDI in hospitals that continues today. Since 1990 in North America, cephalosporins have been licensed for use in food animals. Thus there has been amplification of *C. difficile* in food animals, with subsequent contamination of meat, and vegetables grown in soil containing animal faeces. In some animals such as piglets, there is overt disease with significant impact on industry. Animal strains of *C. difficile* are now infecting humans. *C. difficile* RT 027 probably moved from animals to humans in North America in the early 1990s, initially causing infections in the community at a time when community-acquired (CA) CDI was thought rare. Mutation to FQ resistance at about this time and excessive use of FQs drove RT 027 spread, in North America and later Europe, once it entered the hospital system. This is now occurring with RT 078, another animal strain. *C. difficile* continues to expand in food animal populations, driven by cephalosporin use, and animal strains of *C. difficile* are driving the world-wide epidemic of CA-CDI. It is essential that a One Health approach is used to solve this problem.

INV4

NOVEL REGULATORY MECHANISMS CONTROL SPORULATION INITIATION IN *CLOSTRIDIUM DIFFICILE*

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To survive in aerobic environments and be transmitted from host to host, *C. difficile* must undertake a complex developmental process to form a dormant spore. Evidence suggests that like many spore-formers, *C. difficile* initiates sporulation as cells transition from exponential to stationary growth phase. The entry into stationary phase marks a critical decision point, whereby gene expression shifts to allow cells to produce toxins, obtain more nutrients and transform into dormant spores. Despite the importance of spores for *C. difficile* transmission, very little is understood about the regulatory mechanisms by which *C. difficile* initiates and controls the decision to sporulate. Genome analysis has revealed that *C. difficile* lacks many key genes necessary for spore initiation in other spore-forming bacteria. But, the *C. difficile* genome does encode the master regulator of sporulation initiation, Spo0A. Spo0A activation is a tightly regulated process in spore-forming bacteria because it represents the focal point of initiation. Once Spo0A is expressed and phosphorylated, sporulation-specific gene expression begins. Our data indicate that *C. difficile* has unique genes and regulatory mechanisms to control Spo0A activation that are not found in the early sporulation systems of other bacteria. Here we report on a novel regulatory protein that controls sporulation initiation, as well as toxin expression in *C. difficile*. This protein, which we termed RstA (*i.e.,* Regulator of sporulation and toxins, A), positively influences sporulation by inhibiting the transcription of a repressor of Spo0A activation. In addition, RstA represses toxin production by inhibiting transcription of the alternative sigma factor, SigD, which transcribes the toxin regulator, TcdR. These results provide the first evidence of a mechanism for dual control of sporulation initiation and toxin expression in *C. difficile.*

INV5

TREATMENT OF CDI - PRESENT AND FUTURE

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During the last decade, *Clostridium difficile* emerged as a major enteropathogen. Until recently, two molecules were proposed as a treatment: metronidazole and vancomycin. Over the last few years there was a considerable amount of work focused on the definition of *Clostridium difficile* infection (CDI) to be able to correctly classify the patients based on severity or the potential for recurrence, and at the same time a large number of new therapeutic approaches with new molecules but also more physiopathological hypotheses. From these recent data we can now propose for each clinical situations a specific treatment summarized in the ECCMID guidelines. Basically metronidazole, vancomycin and fidaxomicin were clearly chosen as the reference molecules. Other potential candidates are currently evaluated like cadazolid, rifaximin, nitazoxanide or thuricin. Although interesting on a research point of view, neutralization of cytotoxin does not seem very promising, Tolevamer did not show a potent efficacy compared to vancomycin. Focusing on the immunological hypothesis, immunoglobulins remains marginally indicated with a very low level of proof. Human monoclonal antibodies and toxin directed vaccine data, on the contrary, look very promising; the main problem to solve will be to choose the best patients to indicate these molecules. Beside the pharmaceutical approaches we have a limited number of positive data on probiotics with, to date, no clear indication in CDI. The challenge we will face in the near future will be to find a balance between a pharmaceutical approaches, including the newer molecules, the immunological theory with antibodies and vaccine, and of course the intestinal microbiota transplantation which is associated to very impressive results. CDI remains a highly moving subject with major innovations requiring a major reactivity to adapt our clinical practice.

INV6

PRACTICAL ASPECTS OF FECAL MICROFLORA TRANSPLANTATION (FMT) FOR CDI

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Multiple recurrences of Clostridium difficile infection (CDI) remain frustratingly common in clinical practice following metronidazole, vancomycin or even fidaxomicin as standard treatments. Due to high response rates, fecal microflora transplantation is becoming more widely accepted as the method of choice to arrest the cycle of recurrent Clostridium difficile infection. Donor selection has shifted from family members to treat individual cases to unrelated donors to treat multiple recipients due to costs of donor screening and a shift to hospital programs using 'universal donors'. Whereas donor screening has become more standardized, additional safety testing and monitoring is being considered for commercial application. Longer term follow up for adverse events is also recommended by the FDA. A multinational registry of patients receiving FMT to track long term outcomes is desirable. Health regulators have recently lifted the restrictions to a clinical trial format for provision of FMT so that access to FMT is more widely available. Various methods of delivery of the microbial inoculum into the intestinal tract have all resulted in similar high rates of effectiveness and apparent low rates of adverse events, such that there is now an evolutionary process where the most effective and efficient methodologies are being evaluated. This process is an indirect approach at the discovery of how components of the microbiome contribute to the concept of "colonization resistance" against C. difficile.

FMT variables currently being evaluated include a) prior treatment of CDI to attain control of symptoms and bowel cleansing prior to procedures; b) routes of administration [enema, colonoscopic delivery, nasogastric/duodenal-jejunal tubes, oral capsules]; c) inoculum source/type: unmodified microbes from human donors; modified human donor material to select for components of the microbiome to increase safety and retain effectiveness; d) combinations of selected microbial species grown and harvested in fermentation systems and assembled in various combinations without and with guidance based on serial metagenomic fecal flora studies from samples obtained from fecal transplantation recipients/donor pairs and/or supported by animal model studies; e) inoculum effect based on fresh or frozen storage , volume and microbial density; f) expanded indications for FMT to include inflammatory bowel disease and antibiotic resistant organism carriage in association with concurrent CDI infection. Higher relative costs and complexity for invasive delivery systems would favor oral delivery systems, and which could allow for both therapeutic treatment and repeat treatments as necessary to re-complement the intestinal microbiome, and also to be a platform for preventative strategies following broad-spectrum antibiotic therapy for at-risk patients.

INV7

THE CARROT OR STICK? USING HEALTHCARE-ASSOCITED *C. DIFFICILE* INFECTION RATES AS A PERFORMANCE MANAGEMENT TOOL

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In recent times there has been increased media interest, public and political pressure for healthcare systems to monitor indicators of patient safety and quality, especially healthcare-associated infection (HAI). A variety of infection prevention and control indicators (process, structure and outcome) are used internationally for this purpose with a wide range of reporting strategies. These range from mandatory public reporting with financial penalties if government-set targets are breached to confidential composite process indicator reporting.

Traditionally *Clostridium difficile* infection (CDI) surveillance has been the domain of 'infection experts' including epidemiologists, microbiologists and infection prevention and control practitioners. Detailed surveillance reports would be circulated among experts, however, few non infection experts, specifically healthcare management took note and/or believed that these figures were relevant to them.

HAI rates including healthcare –acquired *Clostridium difficile* infection (HA-CDI) rates are increasingly being used for performance management of hospital senior management teams as indicators of patient safety. In this context, HA-CDI rates are usually integrated into a hospital performance management scorecard or quality dashboard in conjuction with financial and other (e.g. access) indicators. A variety of performance management approaches are used worldwide including public reporting, target setting and financial penalties.

In this presentation the 'carrot' versus 'stick' approach to using HA-CDI for performance management will be reviewed. Using HA-CDI presents opportunities for infection specialists to highlight the importance of CDI as a HAI to those with the financial and political power to implement prevention and control strategies. It is therefore important that key decision makers for healthcare policy and strategy (e.g., healthcare senior management teams, politicians) understand the significance of HA- CDI rates, how to interpret them in conjuction with other infection prevention and control/antimicrobial stewardship indicators and the various caveats in data interpretation. The author will present her experience as the Irish national clinical lead for HAI prevention including lessons learnt from communicating with a range of healthcare managers and senior politicans.

INV8

TRANSMISSION AND FUTURE PREVENTION STRATEGIES

Erik R. Dubberke, MD, MSPH

The two primary strategies to prevent *Clostridium difficile* infection (CDI) in healthcare facilities are to prevent *C. difficile* transmission from patients with CDI and to improve antimicrobial prescribing (in order to decrease risk of CDI if transmission occurs). Although these methods are effective at preventing CDI, there are inherent limitations to these approaches. Limitations to preventing *C. difficile* transmission from patients with CDI include the period of greatest risk for transmission from patients with CDI is the window from onset of symptoms until diagnosis and the need for healthcare worker adherence to contact precautions and environment disinfection. The greatest limitation to preventing transmission from patients with CDI is there are likely more transmission events from asymptomatic *C. difficile* carriers, a population that is currently ignored. Improved antimicrobial prescribing will decrease the number of patients who receive antimicrobials but do not have a bacterial infection. However, approximately 75% of people on antimicrobials do have an infection that requires an antimicrobial, so there will always be a large population of people at risk for CDI in healthcare settings. In addition, efforts to improve antimicrobial prescribing have been modest at best. Fortunately, there are several potential methods to improve upon current prevention practices. One such method would be to prevent *C. difficile* transmission from asymptomatic carriers. Preventing transmission from asymptomatic carriers should decrease new *C. difficile* acquisitions, and thus CDI. There are several potential approaches that may decrease risk of CDI if transmission occurs. One would be to focus antimicrobial stewardship program efforts on infections that account for a large proportion of CDI cases and where antimicrobials are first started. Another approach may be through manipulation of the enteric microbiome of patients who need antimicrobials. A third approach that may soon be available is vaccination against *C. difficile* toxins.

INV9

DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTIONS: WHEN AND HOW?

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Clostridium difficile is recognized as the most frequent etiologic agent of healthcare-associated diarrhoea in hospitalized adult patients. It can also be responsible for antibiotic-associated diarrhoea in nursing home and in the community. The main virulence factors are assumed to be the TcdA and TcdB toxins which both exhibit enterotoxic and cytotoxic activities whereas non toxigenic strains are considered as non-virulent.
The diagnosis of *Clostridium difficile* infection (CDI) is based on clinical presentation and laboratory tests, confirming the presence of a toxigenic strain or toxins in stools. In the context of recent increases in the incidence and severity of CDI worldwide, a rapid diagnosis is essential for optimal treatment and implementation of contact precautions, but continues to be challenging. An accurate diagnosis is also essential to get reliable data for surveillance, in order to assess the efficacy of intervention measures to reduce CDI.
There are currently two reference assays for the diagnosis of CDI with different targets: the cytotoxicity assay which detects free toxins in the stools and the toxigenic culture which detects the organism with the potential to produce toxins *in vitro*. A recent multicenter study has shown that detection of free toxins in patients' fecal samples, compared with detection of a toxigenic strain without detectable toxins in stools, correlates with the disease severity and mortality.
Other tests used for the diagnosis include Enzyme immunoassays (EIA) targeting toxins A and B in stools, Nucleic acid amplification tests (NAATs) and EIA for glutamate dehydrogenase (GDH). EIA are still widely used, but current guidelines recommend that these assays not be used as standalone tests for CDI, primarily because they lack sensitivity. NAATs that target *tcdA* and/or *tcdB* genes are sensitive and rapid, but, like toxigenic culture, may lack specificity because they cannot differentiate between active infection and asymptomatic carriage. GDH is an enzyme produced by both toxigenic and non-toxigenic strains but has a high negative predictive value. To overcome the suboptimal performance of standalone tests, two-step algorithms were recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in 2009. Although it was reported in literature that accurate and rapid diagnostics for CDI are essential for clinical care as well as for national and international CDI surveillance to guide infection control, a consensus on testing practice was not established. More recently, algorithms were categorized by experts involved in revision of the European diagnostics guideline as 'optimal' when they meet the terms of a rapid turnaround time, a high sensitivity and specificity and free toxin detection in the faeces. 'Acceptable' algorithms met the same criteria but without detecting free toxins in the faeces. Other algorithms were designated as 'incomplete'. In Europe, a survey performed in 2014 indicated that 46 % of labs only use an 'optimal' CDI testing algorithm whereas 15% and 40% used an 'acceptable' or 'incomplete' strategy, respectively that could lead to a misdiagnosis of CDI. In addition, in a recent European multicenter point prevalence study where all the diarrheal stool samples were systematically tested for *C. difficile*, 23% of patients with CDI were underdiagnosed by the participating hospitals because of the absence of clinical suspicion.
The wide variation of testing strategy for *C. difficile* and the lack of clinical suspicion can hinder patients' care and skew the epidemiological data, leading to an underestimation of the true burden of the disease.

INV10

CLOSTRIDIUM DIFFICILE VIRULENCE FACTORS

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Clostridium difficile (CD) is the prominent etiological agent of healthcare-associated diarrhoea in adults with an imbalanced gut microbiota. The disease symptoms range from mild diarrhoea to life-threatening pseudomembranous colitis and are mainly due to the two large toxins TcdA and TcdB, which are readily essential for the disease manifestations. However, importance of each toxin seems to differ according to strains and experimental conditions. The role of the additional binary toxin expressed by some strains, among which epidemic 027 strains, is not clearly established, although it has been related to higher morbidity and mortality. This toxin promotes bacterial adhesion to the host by modulating the cell cytoskeleton. After germination, CD vegetative cells are able to adhere to gut tissues. The ensuing dissemination within the gut environment associated with the bacterial resistance to the host response governs the colonisation process, which is undoubtedly a prerequisite for CDI. To date, several putative adhesins have been identified, most of them displaying MSCRAMM function, but studies of adhesin mutants clearly underlined the multi-factorial feature of CD adhesion to the host. Interestingly, a knock-out mutant of a lipoprotein gene (CD0873) lost nearly completely the capacity to adhere to Caco2 cells, suggesting this component to be one of the main CD adhesin. Flagella have also been involved in the colonisation process, but their role depend on the strain tested. In the 630 background, flagella are not essential for virulence and colonisation in animal models. In contrast, the wild type 027 strain R20291 clearly out-competed a flagellin mutant in a competitive dixenic mouse experiment. This difference in strain behaviour is also true regarding the regulation of toxin expression by the flagellar operon: in the 630 strain, it modulates toxin expression probably by the way of the *sigD* sigma factor. Two factors involved in resistance to host innate response have been characterized to date and rely on modification of surface components: deacetylation of peptidoglycan and D-alanylation of teichoic acids, which mediate resistance to lyzozyme and to cationic antimicrobial peptides, respectively. Several studies using knock-out mutants suggest that CD may undergo a switch between the adhesion phenotype and the motility phenotype during the course of infection, regulated by the c-di-GMP intracellular level. In particular, high c-di-GMP levels induced CD aggregation by the mean of type IV pili expression; *in vivo,* this could result in biofilm formation that, associated with persistence of spores, could promote the occurrence of relapses observed in at least 20% of patients.

INV11

ANTIBIOTIC RESISTANCE IN CLOSTRIDIUM DIFFICILE – RECENT ADVANCES

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Antibiotic therapy play a central role in the development of *Clostridium difficile* infection (CDI) and the risk is higher if *C. difficile* strains are resistant to antimicrobial agents used. Antibiotic resistant *C. difficile* isolates are increasing and most of them are currently multidrug resistant (MDR). Beside to the historical resistance to MLSB antibiotics (e.g. erythromycin and clindamycin), resistance to fluoroquinolones and rifamycins have recently emerged and spread in *C. difficile* population. Although the majority of strains is still susceptible to the antibiotics commonly used for the treatment of CDI, higher treatment failure and recurrence rates have been reported. In particular, a rising incidence of *C. difficile* isolates with increased metronidazole MICs has been observed. The presence of *C. difficile* subpopulations with reduced susceptibility to metronidazole in the human intestine may be one of the factors responsible for reduced antibiotic efficacy *in vivo*. Exposure to metronidazole can induce *in vitro* resistance to this antibiotic. This phenomenon has been observed also for vancomycin and fidaxomycin, even if strains with reduced susceptibility to these antibiotics are still rarely found. Mobile genetic elements (MGEs) have a central role of in the acquisition of antibiotic resistance genes and in their persistence and spread among *C. difficile* strains. Together with the well-known Tn*5398* (conferring resistance to MLSB) and Tn*5397* (conferring resistance to tetracycline) several new elements, mostly belonging to the Tn*916* and Tn*1549* conjugative families, have been identified. *C. difficile* shows an inherent ability to acquire MGEs and different mechanisms (conjugation, transduction, transformation) are involved in the transfer of these elements between strains. MGEs can co-localized together forming complex chimeric structures containing not only antibiotic resistance determinants but also other factors that allow the bacterium to adapt challenging environments. Mutations in the antibiotic target genes *gyr* and *rpo*B have been demonstrated responsible of resistance to fluoroquinolones and rifamycins. Preliminary studies *in vitro* also suggest that reduced susceptibility to fidaxomicin and vancomycin could be associated with mutations mediating target modifications. Increasing evidences indicate that antibiotic resistance in *C. difficile* can be multifactorial. *C. difficile* resistance to metronidazole, in which different mechanisms (e.g. alterations of metabolic pathways and biofilm formation) seem involved, is an example of the possible complexity of this phenomenon. The genetic plasticity of *C. difficile* and its propensity to acquire new resistance determinants may dramatically complicate prevention and treatment of CDI. More effective antibiotic stewardship programs, together with a constant monitoring of MIC levels and characterization of strains, are necessary to minimize this risk.

INV12

C. DIFFICILE INFECTION: THE TANGLED WEB OF EPITHELIUM, MICROBIOTA AND PATHOGEN.

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It is clear that the complex community of bacteria that inhabits the human gut plays a key role in maintaining health. Disturbances of the gut microbiota play an important role in the pathogenesis of *Clostridium difficile* infection (CDI). While it is clear that disruption of the gut microbiota by antibiotics underlies the majority of cases of CDI, pathogen characteristics and the epithelial response is also central to the development of colitis. I will review how studies of the dynamics and function of the gut microbiota influences the development of CDI via interactions with the pathogen and the host epithelium.
A key node in the interactions between a host and its gut microbial community is metabolism. The gut bacterial community is integral to the metabolic potential of the host. Disruption of the microbiota can also shift host metabolic profiles towards one that supports the growth of bacterial pathogens such as *C. difficile*. We have shown that communities that are drastically different in terms of membership can provide equivalent resistance to colonization by *C. difficile*. Rather than similarities in the community structure, the commonality between these resistant communities was their metabolic profile. A number of investigators have recognized the central role that bile acid metabolism has in influencing the physiology of *C. difficile* in the intestinal tract. Disruption of the microbiome can shift the bile acid pool to create an environment that supports both the germination of *C. difficile* spores as well as vegetative outgrowth and toxin production.

C. difficile interactions with the microbiota do not occur in a vacuum however. The host epithelium plays a key role in the pathogenesis of *C. difficile* colitis. Until recently, much of our understanding of this role has come from studies of human patients and the use of animal models. We have recently started to use human intestinal organoids derived from pluripotent stem cells to examine epithelium/microbiota and epithelium/pathogen interactions. This system allows us to examine interactions at physical and temporal scales that are impractical in human or animal systems. The combination of microbial ecology, molecular epidemiology and tissue engineering approaches coupled with clinical studies is providing a greater understanding of the etiopathogenesis of CDI that will hopefully lead the way to novel means to prevent and treat this important healthcare-associated infection.