Microbiota predict *Clostridium difficile* severity in germ-free mice colonized with human feces

Running title: Microbiota predict *C. difficile* severity in humanized mice

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Abstract

Clostridium difficile causes diarrheal disease when it successfully colonizes a dysbiotic gut microbial community. Current mouse models to study *C. difficile* infection (CDI) rely on pre-treatment with antibiotics to disrupt the mouse microbiome prior to inoculation. This model does not allow for analysis of human-associated microbial community members that modulate *C. difficile* colonization and expansion. To study human-associated microbes in the context of CDI, we inoculated germ-free C57BL/6 mice with one of 16 human fecal samples from diarrheal or healthy donors and challenged with C. difficile 14 days later. Five unique donor-mice combinations resulted in severe CDI while the remaining 11 only experienced mild disease. Both healthy and diarrheal donors were susceptible to colonization and severe symptoms of CDI. To determine if specific microbes were associated with disease severity outcomes, we built a classification Random Forest machine learning model based on relative abundance data of the communities prior to infection. The model identified a number of bacterial populations associated with the development of severe CDI, including *Bacilliales, Ruminococcaceae, Ruminococcus, Staphylococcus, Streptococcus and Bacteriodetes*. Additionally, a regression model accurately predicted colonization levels of *C. difficile* at one to ten days post-infection. This model explained 99% of the variance in the number of CFU isolated from mouse stool. Members of *Lachnospiraceae, Parabacteroides, Bacteroidales, Bacteroidetes, Porphyromonadaceae* and unclassified *Bacteria* families were predictive of future *C. difficile* colonization levels. Finally, challenging these mice with different strains of *C. difficile* revealed that susceptible human-associated microbial communities were prone to severe disease independent of strain type. Taken together these results suggest that human-associated microbial communities can be recapitulated in germ-free mice and used to characterize dynamics of CDI. Because both healthy and diarrheal patients were susceptible to severe CDI, machine-learning models are useful to identify bacterial populations that allow colonization and contribute to the development of *C. difficile* associated disease in humans.

Introduction

Clostridium difficile is an opportunistic pathogen of the human lower gastrointestinal tract. Disruption of the native microbial community of the gut by antibiotics is the most common risk factor for development of C. difficile infection (CDI) (1). C. difficile is a spore-forming bacteria and can persist on abiotic surfaces and is not readily killed by ethanol-based hand-sanitizers, putting hospital patients particularly at risk. Indeed, ~12% of hospital acquired infections in the United States are due to C. difficile and result in up to 15,000 deaths annually (2).

Murine models to study CDI typically rely on treating conventionally-raised mice with antibiotics either in drinking water or by injection to induce susceptibility (3, 4). This model provides a convenient way to study C. difficile pathogenesis and virulence factors. Numerous microbiome studies have been performed using this model to determine the antibiotic classes (5), starting microbial community (6) and metabolites (7) that impact development and severity of CDI. While informative, these studies are somewhat removed from human disease because they only examine mouse-associated microbial communities.

Gnotobiotic or germ-free mouse models have been used for a range of studies of CDI, including assessment of species-specific interactions between C. difficile and competing microbial community members (8), analysis of nutrient restriction (9), in vivo transcriptomics of C. difficile and examination of host immune response to CDI (10). Further, CDI therapeutics such as antibiotics and fecal microbiota transplants have been tested extensively in a gnotobiotic-piglet or piglet-to-gnotobiotic-mouse model of disease (11), (12). Pigs have a longer digestive tract with components more similar to humans than mice and are typically infected by strains typical in human infection (13). However, the murine and porcine microbiomes typically do not resemble those of the human gut.

The power of the gnotobiotic models to study CDI has been further realized by first inoculating germ-free mice and piglets with human stool microbes. In one study, germ-free piglets were acutely colonized with human feces for one week and then treated with tigecycline. After challenge with C. difficile none of the antibiotic-treated piglets succumbed to infection, while some of the untreated

human-colonized pigs did (11). Further, germ-free mice colonized with human feces were bred over several generations to create a cohort of mice with identical human-derived microbiomes (14). These mice were subsequently treated with a five-antibiotic cocktail to induce dysbiosis and then were successfully colonized by C. difficile (14). While informative, these studies were limited in their use of only one human donor as input inoculum. In order to best understand the impact of C. difficile pathogenesis on human disease, we must have a laboratory model that allows for study of a variety of human-derived microbiomes.

To test the impact of individual human microbiomes on CDI, we colonized germ-free mice with 16 different human stool donors. We then characterized human-associated microbiome response to C. difficile challenge. Additionally, the use of machine-learning models allowed us to build a predictive model that classified “at-risk” microbiomes prior to infection with C. difficile. These findings show that human-associated microbiomes can be at risk for CDI even in the absence of antibiotics and that study of mice colonized with human feces provides a range of clinical outcomes.

Results

Discussion

Materials and Methods

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Figure 1. Number of OTUs sampled among bacterial and archaeal 16S rRNA gene sequences for different OTU definitions and level of sequencing effort. Rarefaction curves for different OTU definitions of Bacteria (A) and Archaea (B). Rarefaction curves for the coarse environments in Table 1 for Bacteria (C) and Archaea (D).

References