

***Klebsiella pneumoniae* isolates from patients with inflammatory bowel disease influences *Clostridioides difficile* pathogenesis**

Introduction: *Clostridioides difficile* is a gram-positive bacterium responsible for half a million infections and associated 29,000 deaths annually in the United States alone. Patients with inflammatory bowel disease (IBD) have increased morbidity and mortality with *C. difficile* infection (CDI). As IBD patients are susceptible to CDI without the use of antibiotics, we sought to determine if bacteria present in the gut microbiota of these patients could be influencing *C. difficile* pathogenesis. We probed the IBD Transcriptome and Metatranscriptome Meta Analysis (TaMMA) database and found that *Klebsiella pneumoniae* was increased in the stool of patients with ulcerative colitis, a subset of IBD. We thus hypothesized that *K. pneumoniae* influences *C. difficile* pathogenesis. **Methods and Results:** We received clinical isolates of *K. pneumoniae* from patients with IBD and utilized those, as well as two commercially available strains of *K. pneumoniae* for experiments. We grew *C. difficile* alone, *K. pneumoniae* alone, or a combination of the 2 species and tested growth with almost 200 nutrient sources using Biolog plates and found that the nutrient sources utilized by the combination of bacteria shifted from both bacteria alone. To test if *K. pneumoniae* was producing a metabolite that was influencing *C. difficile*, we grew *C. difficile* with *K. pneumoniae* conditioned media and measured growth over time. We saw that *C. difficile* growth was enhanced by *K. pneumoniae* conditioned media when compared to unconditioned media. We next tested if *K. pneumoniae* influences *C. difficile* toxin production utilizing LifeAct expressing Vero cells. We saw that *C. difficile* grown with *K. pneumoniae* conditioned media had enhanced toxin production, as seen by increased Vero cell rounding when compared to Vero cells treated with *C. difficile* grown in unconditioned media. To determine how *C. difficile* and *K. pneumoniae* affects the intestinal epithelium, we treated inside-out colonic organoids with *C. difficile*, *K. pneumoniae*, or the combination of the two bacteria before collecting the organoids for RNA sequencing. We saw that the combination of bacteria caused decreased expression of *Muc2*, as well as increased expression of *Tnf*. **Conclusion:** These results suggest that *K. pneumoniae* influences *C. difficile* growth and pathogenesis. *K. pneumoniae* is found in low levels in the healthy human gut, however, patients with IBD have increased abundance of *K. pneumoniae*, which could potentially help drive *C. difficile* fitness and colonization. Therefore, we believe that clarifying the mechanism by which *K. pneumoniae* is interacting with *C. difficile* will help to better understand CDI in patients with IBD.