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Klebsiella Pneumoniae in the Colonic Mucus Layer Influences *Clostridioides Difficile* Pathogenesis

Background: *Clostridoides difficile* is an intestinal pathogen responsible for 500,000 infections and 30,000 deaths annually in the US. Intestinal microbiota disruption allows *C. difficile* to colonize the colonic mucus layer; however, the mechanisms by which other microbes influence *C. difficile* remains unclear. Using stool-seeded bioreactors with mucus-coated inserts, we identified *Klebsiella pneumoniae* in the mucus-associated microbiota with *C. difficile*. **Hypothesis:** We therefore hypothesized that *K. pneumoniae* promotes *C. difficile* intestinal colonization. **Methods & Results:** Analysis of 77 *C. difficile* genomes revealed that no *C. difficile* strains harbored the glycosyl hydrolase (GH) families required for mucin degradation. In contrast, 274 *K. pneumoniae* genomes harbored at least 3 mucin-degrading GH families. To confirm the ability of *K. pneumoniae* to degrade mucus, we grew 11 strains of *K. pneumoniae* in a fully defined bacteria media lacking glucose, supplemented with porcine intestinal MUC2 mucus. All strains exhibited robust growth, indicating that *K. pneumoniae* can enzymatically degrade mucus and use it as a primary carbon source. Under the same growth conditions, *C. difficile* was unable to grow. However, *C. difficile* was able to use freely available mucin glycan oligosaccharides (sialic acid, fucose, galactose, GluNAc, and GalNAc) when supplemented into glucose free media. Growth curve revealed that cell-free supernatant from *K. pneumoniae* strains enhanced the growth of *C. difficile* strains, suggesting that *K. pneumoniae* could enhance *C. difficile* growth. Moreover, time-lapse microscopy of LifeAct expressing Vero cell rounding revealed that *K. pneumoniae* cross-fed *C. difficile* and suppressed toxin production. Select *K. pneumoniae* strains produce substantial biofilm. To examine biofilm production, we grew *C. difficile* with *K. pneumoniae* for three days and stained with crystal violet. Interestingly, we observed that some, but not all combinations of *C. difficile* with *K. pneumoniae* increased biofilm production when compared to *C. difficile* alone. Finally, we examined how *K. pneumoniae* impacts *C. difficile* infection *in vitro* using colonic organoids by RNA sequencing. **Conclusions:** These results suggest that *C. difficile* and *K. pneumoniae* interact with one another, which impacts the pathogenicity of *C. difficile.*