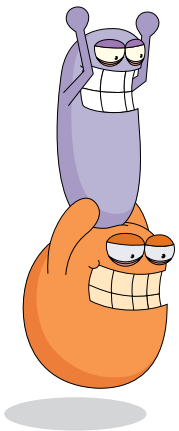
Bacterial pathogenesis

Helping *C. difficile* to thrive

The microbial environment in the gastrointestinal tract is rich and dynamic, and it influences the establishment of infections by enteric pathogens. Enterococci are a group of opportunistic pathogens that are often associated with the important enteric pathogen *Clostridioides difficile*. How these two bacteria interact and how *Enterococcus* species influence *C. difficile* infection (CDI) are currently unknown. Now, Smith et al. report that expansion of enterococci (*Enterococcus faecium* and *Enterococcus faecalis*) in the gut reprogrammes *C. difficile* metabolism, thus enhancing its pathogenesis.

The authors first infected enterococci-depleted mice with *E. faecalis* before CDI and showed that enterococci support *C. difficile* colonization. Using fluorescence in situ hybridization (FISH) during CDI in mice, coupled with biofilm in vitro assays, the authors show that *C. difficile* and *E. faecalis* form a biofilm together, which enhances *C. difficile* survival. The authors subsequently analysed the genomes of clinical *C. difficile* and vancomycin-resistant *E. faecium* and *E. faecalis* isolates from co-colonized patients and demonstrate that mobile genetic elements can transfer between the two species during infection.

Next, the authors explore the association between enterococci and CDI in closer detail. They showed that mice co-infected with both bacteria presented higher *C. difficile* toxin titres in faeces than mice infected only with *C. difficile*. In a cohort of human adults with



CDI, the relative abundance of *Enterococcus* species was positively correlated with metrics of severity during CDI, and, in vitro, enterococci increased *C. difficile* toxin production.

So how do enterococci and *C. difficile* interact at the molecular level? To understand this, the authors performed transcriptomic and metabolic network analyses, and show that *Enterococcus* species remodel the metabolic environment in the gut by providing fermentable amino acids (D-ornithine, L-alanine, L-leucine and L-valine) to *C. difficile* and depleting arginine during growth.

The arginine deiminase (ADI) pathway modulates arginine catabolism and ornithine export via the arginine–ornithine antiporter ArcD. The authors used an *E. faecalis* ArcD transposon mutant to show that arginine depletion mediated by the ADI system is key to enhance *C. difficile* virulence. The *arcD* gene was found across enterococcal strains, as well as in other taxa such as *Lachnospiraceae* and *Eggerthellaceae*, suggesting a widespread capacity of the microbiota to impact *C. difficile* virulence. Through a set of in vivo experiments where germ-free mice were co-infected with *C. difficile* and wild-type or *arcD*-mutant *E. faecalis* strains, the authors confirmed the role of arginine and ornithine in CDI.

Lastly, they analysed the faecal metabolome of children with inflammatory bowel disease and CDI, and show a positive correlation between *C. difficile* and ornithine, supporting a role of fermentable amino acids in CDI.

> **"expansion of enterococci ... in the gut reprogrammes *C. difficile* metabolism, thus enhancing its pathogenesis"**

Overall, these findings provide mechanistic insights into the interaction between *C. difficile* and *E. faecalis* in the gut, where *E. faecalis* enhances *C. difficile* fitness and virulence by regulation of key amino acids via the ADI pathway. Further studies are warranted to fully understand whether these mechanisms are employed by other members of the microbiota, and their impact on CDI.

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Viral infection

Across the mucus

SARS-CoV-2 enters the epithelial cells in the upper respiratory tract to begin replication and infection. Nasal airways are composed of stratified multiciliated epithelial cells and mucus-producing goblet cells. How the virus overcomes this mucus barrier to infect the nasal epithelium is not completely understood. In this new study, Wu et al. show that first, SARS-CoV-2 binds the ACE2 receptor present on airway motile cilia. Cilia then mediate the transport of the virus across the underlying mucus–mucin protective barrier. Once SARS-CoV-2 accesses the basal cell body, it manipulates the host cell machinery to induce the activation of p21-activated kinases 1 (PAK1) and 4 (PAK4). Such reprogramming results in the elongation and branching of microvilli, which enable the virus to reach the nasal airways and disperse via mucus flow. Finally, the authors show that Omicron variants bind with higher affinity to the cilia and show accelerated entry compared with other variants, which explains their higher transmissibility. In sum, SARS-CoV-2 interaction with cilia and microvilli is key for viral replication and spread in the airways.

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