Aquaporin-1 and Carbonic Anhydrase II Suppressed by *Clostridioides difficile* Infection and Restored by Faecal Microbiota Transplantation Regulate Gut Barrier Permeability

Introduction:
Clostridioides difficile infection (CDI), the leading cause of hospital-acquired diarrhoea, is primarily driven by two potent toxins, TcdA and TcdB. However, the molecular mechanisms underpinning toxin-mediated disruption of intestinal barrier function and the role of fluid and ion exchange regulators in intestinal permeability during CDI, and their modulation by faecal microbiota transplantation (FMT) remain incompletely understood.

Methods:
Caecal tissues from mice infected with *C. difficile* and treated with or without FMT were analysed via RT-qPCR for expression of aquaporins (AQP1, AQP4, AQP8, AQP11), carbonic anhydrases (CAI, CAII, CAIV), and ion transporters (ANO1, CLCA1, SGLT1, SLC9A3, SLC26A3, SCNN1A, SCNN1B, SCNN1G). Their expression was also validated in colonic biopsies from CDI patients and healthy individuals and primary human colonoids treated with TcdA and TcdB. AQP1, AQP11, CAI, and CAII were knocked down in Caco-2/TC-7 cells by means of shRNAs, and epithelial barrier formation was assessed by transepithelial electrical resistance (TEER) and tight junction immufluorescence in response to TcdA and B.

Results:
CDI significantly downregulated AQP1, AQP11, CAI, CAII, and CAIV expression in murine models, an effect reversed following FMT. Knockdown of AQP1 and CAII in Caco-2/TC-7 cells impaired barrier function, with further deterioration upon TcdB exposure. Validation in human CDI colonic tissues confirmed reduced AQP1 and CAII expression. AQP1 expression was directly suppressed by TcdB in primary human colonoids.

Conclusions:
We demonstrate that AQP1 and CAII are key regulators of gut epithelial barrier integrity during CDI. Their downregulation by *C. difficile* contributes to increased intestinal permeability, a hallmark of disease pathogenesis and their restoration by FMT suggests a potential mechanism for its therapeutic efficacy. These findings identify AQP1 and CAII as promising therapeutic targets for the restoration of barrier function in CDI.