Identifying novel antimicrobials with anti-Clostridium difficile defence properties in human “mini-guts” using microbiological, proteomic and transcriptomic tools

Section 1 – Project Details:

Rationale:  
*Clostridium difficile* infection (CDI) is amongst the most serious healthcare complications that impacts hospitalised and increasingly community populations worldwide. Over 500,000 new cases of CDI occur each year in the US\(^1\) and estimates suggest that greater than 400,000 cases occur annually in Europe.\(^2\) Infection of the colon with this Gram-positive, spore-forming and pro-inflammatory toxin producing anaerobe is potentially life-threatening, especially in the elderly and in patients who have dysbiosis of the gut microbiota following antimicrobial drug exposure. Current antibiotics, while successful in treating the initial infection, are less effective at preventing recurrence and managing severe CDI. Treatment of CDI is complicated by the fact that antimicrobial resistant *Clostridium difficile* isolates are increasing with the US CDC declaring *Clostridium difficile* an Urgent Antibiotic Resistance Threat. There is an unmet need to develop novel alternative therapies to treat this recalcitrant infection. Moreover, in recognition of the fact that *Clostridium difficile* animal testing has only modest predictive value for how pharmaceuticals behave in humans, physiologically relevant models are required for informing the design of more effective and personalised preventative and therapeutic measures against CDI.

Primary intestinal epithelial culture systems (mini-intestines) provide a unique opportunity in which to model infections with enteric pathogens\(^3\)-\(^4\) and are being increasingly used as a valuable preclinical drug screening platform.\(^5\) *Ex vivo* human intestinal preparations are either developed from stem cell-containing intestinal crypts, isolated from small or large intestinal tissues (termed enteroids and colonoids respectively), or induced pluripotent stem cells (termed intestinal organoids). Whereas the former system more closely recapitulates adult intestinal architecture, the latter more precisely resembles human fetal gastrointestinal tissue. As such, enteroids and colonoids may be better positioned to predict drug toxicity and human efficacy more accurately than other models.

This project closely aligns with the lead supervisor’s Anne McLaren Fellowship. Here the focus is on studying the biological activity and anti-microbial potential of a small library of natural pure compounds used in traditional Indian and Chinese medicine. These selected compounds have demonstrable antibacterial, anti-diarrhoeal and anti-inflammatory activity (berberine,\(^6\)-\(^7\) evodiamine\(^8\)), and will be tested alongside their novel semi-synthetic derivatives, in addition to promising new antibiotics with proven anti-*C. difficile* potential developed by the Chan Group (LY256) and newer agents (teixobactin analogues), to inhibit *Clostridium difficile* growth and toxin production. These compounds will also be evaluated for their potential for suppressing the infection-related inflammatory phenotype (Figure 1) in human colonoids. This work will enable us for the first time to move towards the development of a human CDI organoid expression profile that could find utility in developing and optimising personalised anti-*C. difficile* therapies.
Figure 1. Differences in key cytokine mRNA profiles after *C. difficile* toxin challenge in human colonoid cultures. Basolateral exposure of human colonoids to highly purified whole *C. difficile* toxin A and/or *C. difficile* toxin A in combination with *C. difficile* toxin B, but not toxin B alone, induces a dose-dependent increase in pro-inflammatory TNF-a, IL-23 and IL-18 mRNA expression.

**Aims and methodology:**

1. To determine the antimicrobial effectiveness of novel teixobactin analogues, selected natural pure compounds and their novel derivatives with standard Western antibiotics on *C. difficile* bacterial growth and toxin production *in vitro* using broth microdilution assays. This work will be initially primed by our 15 week Wellcome Trust DTP UoB PhD student.
2. To generate human colonoids using primary intestinal biopsies from patients undergoing routine endoscopy and/or surgical resection specimens. This methodology is already established. Biopsies will be derived from healthy and inflamed/diseases tissues in patients with and without CDI. This system will be validated using LY256* and vancomycin (two proven anti-*C. difficile* antimicrobials; *unpublished in vitro and in vivo data from Chan lab*)
3. To determine the effects of novel teixobactin analogues, selected natural pure compounds and their derivatives (those showing most promising anti-*C. difficile* potential in 1) on epithelial barrier function, intestinal gene expression, intestinal stem cell niche and inflammation in infected/uninfected organoid cultures. We will biobank colonic organoid cell lysates and RNA from these experiments for future proteomic and transcriptomic studies (Figure 2).
FIGURE 2. Diagrammatic representation of mini-gut work streams
Benefits and suitability as a PhD project:

The studentship will be hosted by the newly £23.6M funded NIHR Nottingham Biomedical Research Centre, which enjoys strong Gastroenterology, microbiology/infection, stem cell biology and immunology themes. The student will benefit from working closely with a highly dynamic and committed group of scientists interested in infectious disease modelling and the development of novel next-generation anti-microbials. The PhD student will attend and present at fortnightly lab meetings, weekly supervisory meetings, journal clubs, seminars held by the BRC and bi-annual Divisional/School meetings. When appropriate, the student will present their work at national and international conferences and submit it for publication. This project which would span three Schools in the University, in cooperation with international academic partners (Figure 3), would allow the student to acquire a first rate training in a world-leading research environment, leading to a successful early career scientist with all the requisite skills to secure a post-doctoral position in a leading lab.

FIGURE 3. International project research partners
Key References:

Section 2 – Training Provision:

The student will be immersed in a highly collaborative and multidisciplinary group comprising experts in C. difficile micro- (Cockayne) and immunobiology (Monaghan and Robinson), organoid culture (Hannan), and chemical biology/drug testing/’omics’ (Chan and Zhu). The student will be primarily based in the Centre for Biomolecular Sciences where he/she will align with pre-existing research staff employed on Hannan-Monaghan Wellcome Trust, BBSRC, CHAIN Biotechnology and Anne MacLaren Fellowship-funded projects. The student will be trained in several cutting edge cell and molecular biological techniques. These comprise anaerobic culture and broth microdilution assays, primary cell culture including 3D cell culture, cellular differentiation, cell staining (immunohistochemistry and immunofluorescence), light and fluorescent microscopy (including confocal), qPCR, western blotting, ELISA and multiplex assays. The student will receive weekly supervisory meetings with Monaghan and relevant co-supervisors. Excellent opportunities exist for the student to be able to communicate their research findings to the local lab group(s) at CBS, NDBRRC and School of Pharmacy as well as regional and national meetings.
The student will enrol in the FHMS n-Trans PhD programme and also acquire SoM credit values as part of specific taught modules. The student will have the opportunity to undertake training in research methods, Good Clinical Practice, Ethics and enrol in the Graduate School Researcher Development Programme providing transferable skills. This training package will provide a unique and multi-disciplinary training and wide skill portfolio, and will equip the student with an interdisciplinary core provision, cross-cutting from microbiology, immunobiology, and stem cell biology to proteomics and transcriptomics.