Poster #1

Microbiome-based interventions for the control of foodborne pathogens in chickens: What, When and How.

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Abstract

Foodborne pathogens such as *Salmonella* and *Campylobacter* remain significant public health problems in the poultry industry. Although vaccination is beneficial in egg production and breeders in control of salmonellosis, no effective vaccine is available for *Campylobacter* and the young age of slaughter of broiler (meat) chickens presents a barrier to the use of vaccines as does cost. As such microbial based interventions to limit intestinal colonisation of these pathogens is seen as attractive.

Probiotics and microflora-based competitive exclusion products for use in chickens have largely been developed empirically and are usually delivered following placement on farms via feed or water. Their efficacy is mixed. A further complication is that without maternal contact, the early microbiomes of chicks are dominated by taxa that have deleterious effects on gut health. We have used a caecal microbiome transplant to successfully reduce infection and transmission of *Salmonella* and *Campylobacter* in broiler chicks. Through analysis of transplant material and caecal microbiome of transplanted chicks we are able to identify taxa associated with a beneficial phenotype. Through culture, stability testing and ability to colonise the gut we identified three candidate *Lactobacillus spp.* which reduced *Salmonella* colonisation when delivered as a single dose consortium.

Using this platform we also looked at the effect of age and found that transplant administration within 72h of hatch was effective, though delivery within the first 24h is optimal. We also were able to show that delivery by spray or gel drop vaccine delivery systems was effective, opening up the potential of microbiome modification within the hatchery.

Poster #2

IMPACT OF HEAT-KILLED LACTOBACILLI ON MICROBIOTA COMPOSITION AND BEHAVIOUR

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Abstract

Anxiety and depression are the most common mental disorders, however with treatments remaining still limited in their effectiveness by severe side effects. Recently, it has been shown that use of microorganisms and their derivatives may help in diarrhoea, colitis, and respiratory infections and reduce inflammation; nevertheless, their effects on mental health disorders remains limited to few studies demonstrating that supplementation with inactivated Lactobacillus gasseri CP2305 may be beneficial for mental state, sleep quality, and gut microbiota composition in healthy adults under stressful conditions. Therefore, the main goal of this study was to investigate the effect of feeding a heat-killed fermentate generated by two Lactobacillus strains (ADR-159), on the behaviour and microbiota composition of a standard adult mouse.

In this study, 24 eight-week old male C57BL/6 mice were given a diet supplemented with 5% ADR-159, a heat-killed fermentate generated by two strains, Lactobacillus fermentum and Lactobacillus delbrueckii, over a period of eight weeks. After three weeks of intervention, animals were subjected to behavioural testing, and fecal samples were collected for 16S rRNA analysis of microbiota.

Overall, results of the recent study have shown that a prolonged consumption of a heat-killed Lactobacillus fermentate within the diet had no adverse effect on general health, and may bring a favourable behavioural effects demonstrated by increased sociability and lower baseline corticosterone levels, with subtle significant changes in the microbiota composition observed as an increased abundance of Prevotella, followed by reductions in Alistipes and Odoribacter species.

Poster #3

Gastrointestinal Pathogens Detected in Diarrheal Stool Samples from Young Children in Southern Ethiopia

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Abstract

Background: Diarrhoea causes significant morbidity and mortality in children under five in Sub-Saharan Africa. However, due to lack of resources enteric pathogens are not routinely identified in infected patients preventing accurate monitoring of pathogens circulating in specific populations and prescribing of relevant treatments.

Aim: We aimed to identify the causes of childhood diarrheal disease in Wolaita Zone in Southern Ethiopia.

Methods: 321 diarrheal stools from children under five in Wolaita Zone were cultured on selective medium for Campylobacter. 116 stool samples were tested using the EntericBio® Dx real time PCR kit (Serosep) for bacterial and parasite infection. 24 stool samples were tested by multiplex PCR for viral pathogens.

Results: Campylobacter the commonest cause of bacterial gastroenteritis in the western world was not isolated from any stool. DNA was detected in stool from Campylobacter (5%), Shigella (9%), VTEC (2%), Giardia (17%) and Cryptosporidium (7%). Bacterial and parasite co-infections were detected in 5% of samples. 17/24 (70.8%) samples tested positive for viruses. Rotavirus detected in 11/24 samples, was the commonest virus detected followed by Norovirus and Astrovirus (6/24 each), Sapovirus (4/24) and Adenovirus (2/24). More than one virus was detected in 7/24 stools and in 5/24 bacteria and/or parasite DNA was detected also

Conclusion: Results suggest that Campylobacter is not a common cause of childhood diarrhoea in this region, but viruses may be a common cause. The presence of co-infections on diarrhoea severity needs to be assessed. These results have important implications for the treatment of childhood diarrhoea in Wolaita.

Poster #5

Characterising the Potential Therapeutic Effects of Indane Dimers against Chronic Gut Inflammation

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Abstract

Background: Previous research has optimised a compound known as PH46A and its enantiomer PH45 from an indane scaffold used within traditional Taiwanese medicine. The anti-inflammatory properties of PH46A against IBD have been identified however the underlying mechanism for this effect is yet undetermined. Here we investigated the anti-inflammatory nature of Indane dimers within an in vitro setting, assessing impact inflammatory responses in epithelial and macrophage cell lines.

Methods: Using both the Alamar blue and TEER assay we first assessed the effect of pre-treatment with indanes on cell viability and intestinal epithelial integrity in a Caco2 cell line. Pre-treatment of the Raw Blue reporter cell line with indanes prior to exposure to TNF-α was used to assess any manipulation of the NFκB pathway. Furthermore, gene expression of signalling components involved in the TNF-α – NFκB pathway were investigated through qRT-PCR. Finally, the Griess assay was performed to assess manipulation of nitric oxide levels.

Results: Following incubation with both compounds, an increase in cell viability and TEER was observed. Treatment also attenuated NFκB activation in response to TNF-α. Furthermore, this was accompanied by downregulation of elements involved in TNF-α signalling pathways. In addition, pre-treatment with the indanes led to reduced nitric oxide levels.

Conclusion: PH46A and PH45 can exert an anti-inflammatory effect on immune cells through interference with nitric oxide production and the TNF-α - NFκB pathway. Both compounds also provide strength to the intestinal epithelial barrier. These findings reinforce prior observations of an anti-inflammatory potential for indane dimers in the treatment of IBD.

Poster #6

"Antibiotic susceptibility testing of microbial isolates from soil samples taken from the riparian buffer zone of an Irish Dairy farm"

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Abstract

Background:

Farms can be a source of antimicrobial resistance (AMR), with animal manure present in agricultural runoff being one main route AMR can enter the environment. Riparian buffer zones (RBZ) are areas of vegetation that intercept agricultural runoff in cooperation with soil microorganisms like rhizobia, therefore improving water quality. There is a lack of knowledge about bacteria recovered from soil as a source of AMR or potential new sources of antimicrobials.

Methods:

Soil samples were taken from the RBZ of a Dairy farm using a tubular sampler. Samples were serially diluted to 10-6 with PBS, spread on agar plates and incubated at 37°C for 24 hours. Single colonies were then streaked on agar plates and incubated at 37°C for 24 hours.

For the antibiotic susceptibility test, 16 samples were assessed. Single colonies from each plate were incubated in Mueller Hinton Broth for 16 hours at 37°C. Cultures were then diluted to 0.5 McFarland standard using PBS and spread on Mueller Hinton Agar plates. Eight antibiotics were placed on the plates, incubated at 37°C for 16 hours and the diameter of the inhibition zone (mm) was measured.

Results:

Three samples were fully resistant to ampicillin, 7 to aztreonam and 3 to both aztreonam and nalidixic acid. Interestingly, no samples were resistant solely to nalidixic acid.

Conclusion:

Bacteria isolated from soil showed resistance to ampicillin, aztreonam and nalidixic acid.

Poster #7

Immunomodulatory properties and protective effects of a naturally-derived β-glucan in gastrointestinal inflammation

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Abstract

β-glucans are complex polysaccharides occurring naturally as cell wall components in cereals, algae, fungi and bacteria. β-glucans are known to have immunomodulatory functions and may both directly (via pattern recognition receptors) and indirectly (via the microbiota) influence the immune system. There is evidence to suggest that β-glucans demonstrate anti-inflammatory capability in mice exposed to chemically induced colitis. However, structural and chemical differences resultant from the source of the β-glucan, affect their immunomodulatory abilities. The aim of the present study is to investigate a naturally-derived β-glucan (BG) to determine it's immunomodulatory potential, with a particular focus on protective benefits in gastrointestinal inflammation.

Caco2 cells were used to investigate BG -induced transcriptional changes in intestinal epithelial cell barrier markers in response to stimulation with TNF-α. BG increased transcription of protective intestinal barrier-related genes (Occludin and EGFR) and decreased IL-1β transcription in these cells.

Mice were orally administered BG prior to exposure to dextran-sulfate-sodium (DSS; 2.5%) colitis in order to determine protection from disease. Mice who received pre-treatment with BG displayed a trend for lower disease scores throughout DSS administration. A decrease in gut barrier infiltration measured by FITC dextran translocation and a decrease in myeloperoxidase activity in the distal colon were also recorded in these mice.

From these data BG appears capable of reducing disease activity in the DSS model of chemically induced colitis. This is likely due to a combination of anti-inflammatory activity as well as a boost in intestinal barrier integrity.

Poster #8

The mechanistic impact of miR-21 on intercellular junctions and susceptibility to intestinal inflammation

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Abstract

Inflammatory bowel disease (IBD) is an autoimmune disease of chronic inflammation along the gastrointestinal (GI) tract. In the gut, intestinal epithelial cells (IECs) react to changes in the gut environment which typically impact the abundance of gut microbes, thus affecting gut permeability via intercellular junctions, mucus layer amelioration, and enhancing host defenses via synthesis of antimicrobial peptides. These tactics can be regulated by microRNAs, short nucleotides that bind to target mRNA and impact protein synthesis. Of focus to this project is microRNA-21 (miR-21), an oncomiR that is highly upregulated in IBD and has previously been shown to target epithelial tight junctions like occludin and Zo-1 thereby increasing epithelial barrier permeability. However, the molecular mechanisms underlying this phenotype extending from barrier permeability via intercellular junctions remain inconclusive. We show in vivo that both basal and dextran sulfate sodium (DSS)-inflamed colonic tissue from constitutive knockout mice (miR-21-/-) have greater expression levels of several tight junctions (Cldn3, Cldn4, Cldn7, Cldn15, Zo-3) and adherence junctions (Cdh1) compared to wild type controls. Additionally, these were upregulated in other murine intestinal regions, and in vitro via TNFα-induced upregulation of miR-21 in Caco-2 cells which associated with a downregulation of these junction genes. Further, expression of the primary intestinal adheren Cdh1 (E-cadherin) was inhibited by the addition of artificial miR-21while alleviated with anti-miR-21 inhibitor. Importantly, this comparison will determine the impact of miR-21 expression on the gut barrier through regulating junctional complexes and the development of inflammation.

Poster #10

Postbiotics Effect on the Intestinal Mucosa and Macrophage Function

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Abstract

Postbiotics are defined as bioactive compounds produced by the microbiome. They contain by-products like metabolites that can play a role in many biochemical pathways modulating the host immune system and regulating the gut microbiome. Metabolites and other by-products in the gut lumen meet intestinal epithelial cells and resident macrophages and can alter their immune function. The specifics of these pathways, while crucial to homeostasis in the gut, are poorly understood. This project investigates the potential for new therapeutic postbiotic compounds and their ability to modulate host macrophage function. We measured different pro- and anti-inflammatory biomarkers in macrophage cells exposed to the metabolite and postbiotic treatments as well as controls. Preliminary qPCR data suggests metabolite and postbiotic treatments modulate macrophage function. A reduction in TNF-a expression in the postbiotic-treated macrophages suggests an anti-inflammatory effect taking place. This is promising, as inhibition of pro-inflammatory markers such as TNF-a is one of the main effects of postbiotics on the market today.

Poster #12

Interactions between medications and the gut microbiome in inflammatory bowel disease

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Abstract

In view of the increasing evidence that commonly prescribed, non-antibiotic drugs interact with the gut microbiome, we re-examined the microbiota variance in inflammatory bowel disease (IBD) to determine the degree to which medication and supplement intake might account for compositional differences between disease-subtypes and geographic location. We assessed the confounding effects of various treatments on the faecal microbiota composition (16S rRNA gene sequencing) in persons with Crohn's disease (CD; n=188) or ulcerative colitis (UC; n = 161) from either Cork (Ireland) or Manitoba (Canada) sampled at 3 time points. We confirmed the overall trends of significant differences of microbial composition and diversity across IBD-subtypes and geographic locations from our previous study. Treatments explained more microbiota variance than all other factors combined and more than half of the tested medications and supplements showed significant associations with at least one taxon in the gut microbiota. The medication profiles between patients with UC and CD and from different countries varied in number and type of drugs taken. While treatments accounted for a relatively small proportion of the geographic contribution to microbiome variance between Irish and Canadian patients, additive effects from multiple medications contributed significantly to microbiome differences between UC and CD. The highly variable medications profiles of persons with IBD and their effect on the faecal gut microbiota likely impede the discovery of a universally valid microbial signature distinguishing the IBD-subtypes and also, at least in part, explain the high disparity between different IBD microbiome studies.

Poster #13

Modulation of allergic immune responses by human respiratory tract bacteria.

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Abstract

Asthma is a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness, bronchoconstriction and airflow obstruction. In patients with allergic asthma, exposure to inhaled allergens leads to excessive activation of an inflammatory response in the lungs typically mediated by Type-2 immune responses, IgE production and eosinophil recruitment. Increased burden of certain bacterial species, including the Firmicutes *Staphylococcus aureus* and *Streptococcus pneumoniae*, have been associated with disease incidence by next generation sequencing of bronchoalveolar lavage (BAL) and sputum samples from asthmatic patients. Using a murine model of allergic airway inflammation (AAI), we have found that respiratory tract colonisation with *S.aureus* ameliorates airway inflammation in SPF mice, including decreased Th2 cytokine responses and reduced eosinophil accumulation in the bronchoalveolar space. This was associated with increased expression of IFNγ and IL-10 in BAL fluid of colonised mice, and enhanced allergen-specific IFNγ responses by CD4 T cells from the lungs of colonised mice. In vitro, we found that *S.aureus* and *S.pneumoniae* stimulates expression of the Th1-polarising cytokines IL-12p70 and IL-18 by dendritic cells and macrophages, and drive high expression the anti-inflammatory cytokine IL-10 predominantly by macrophages. This data indicates that *S.aureus* and *S.pneumoniae* could stimulate alveolar macrophages to engender a suppressive environment in the lungs that dampens inflammation in the airways and protects against AAI. Reactivation of allergen-specific CD4 T cells by *S. aureus*-stimulated innate immune cells may also skew the immune response to inhaled allergen towards an IFNγ-producing Th1-type response that corrects Th2-driven inflammation and dampens the development of allergic asthma.

Poster #14

Streptococcus pneumoniae infection drives macrophage iron loss in chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterised by chronic bronchitis and emphysema, typically smoking-induced, and the fourth leading cause of death in Ireland. Acute respiratory infections are a significant cause of mortality in COPD, with underlying host factors contributing to such poorly understood. Individuals with COPD display increased levels of iron in lung-resident alveolar macrophages, with iron loading increasing with disease severity. While iron is central to numerous host cellular processes, iron acquisition is also essential to bacterial pathogenicity; however, the consequences of this iron loading on host-pathogen interactions in the COPD lung remains unclear.

Using *Streptococcus pneumoniae*, one of the most commonly cultured pathogens from the COPD lung, we show that infection of a bone marrow-derived macrophage cell line in vitro causes rapid intracellular total iron and heme loss as determined through graphite furnace-atomic absorption spectrometry and a fluorescent heme assay, respectively. This loss isn't accompanied by a corresponding increase in extracellular iron or RNA expression of cellular iron exporters, suggesting a pathogenic bacterial mechanism exists to thieve macrophage iron. Macrophage iron loading through ferric ammonium citrate treatment further exacerbates this phenotype and associates with increased numbers of internalised bacteria, while iron depletion using the chelator deferiprone is protective. Infection of an alveolar macrophage-like cell line yielded a similar loss of iron, indicating this phenomenon is observed in the lung. Characterising the role of iron in this host-pathogen tug-of-war will inform treatment both in COPD and across disorders characterised by susceptibility to recurrent infection.

Poster #16

Comparing the motility of Type I and Type II Bovis group streptococci in response to metabolic conditions that mimic the human gut.

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Abstract

Streptococcus gallolyticus subsp. gallolyticus (Sgg), a Type I Bovis group streptococcus is an opportunistic pathogen that can colonise colorectal cancer tissues and, following colonisation, translocation occurs through the intestinal barrier into the bloodstream. Sgg were previously believed to be non-motile, however, we have shown that Sgg are motile bacteria, that may utilise type IV pili for twitching motility. Following this discovery we were interested in discovering whether Type II bovis group streptococci that are not associated with colorectal cancer and yet still can cause bloodstream infections, are also motile. We also wanted to know how they respond to different monosaccharides present in the human gut and different temperatures with respect to motility and colonic epithelial cell invasion.

The novel motility phenotype is abolished in Sgg in response to growth in different monosaccharides. In addition a reduction in invasion of colonic epithelial cells by Sgg is observed when glucose is present. This may correspond to increased cell invasion in cancerous tissues where glucose is limited. However Type II Bovis group streptococci, S. pasteruianus and S. lutetiensis also display motility that responds to the presence of monosaccharides.

Based on the results from this study, we hypothesise that the response of bovis group streptococci to changes in temperature may be a factor that discriminates the motile behaviour of Type I and Type II bovis group streptococci and could provide a tool to manage Sgg infections in colonic cancer patients.

Poster #17

Effects of a postbiotic consisting of heat-treated lactobacilli fermentate on mouse small intestinal ion transport and motility *ex-vivo*

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Abstract

Lactobacillus LB is a heat-treated preparation of cellular biomass and fermentate (postbiotic) which alleviates acute diarrhoea and ameliorates symptoms of irritable bowel syndrome in human studies. We investigated whether modulation of intestinal ion transport and motility underpins the beneficial effects of this postbiotic observed clinically.

A low-lactose preparation of the postbiotic Lactobacillus LB (LLL) was applied luminally to Ussing chambers and into vertical organ bath setups to assess its effects on naïve small intestinal tissue from C57Bl/6 mice. Both were carried out as described previously.

LLL (5%) significantly increased baseline short circuit current (I_{SC} [$\mu A/cm^2$]) compared to Krebs-control (51.2 [37.6 – 64] versus -2.4 [-9.6 – 6.4], N = 6, $p < 0.05$, median [IQR]). This effect was insensitive to pre-treatment with the neurotoxin TTX (vehicle-LLL, 40 [31.2 – 52] versus 300nM TTX-LLL, 34.4 [25.6 – 60.8] or a Na-K-2Cl co-transporter inhibitor (vehicle-LLL, 50.4 [28.8 – 66.4] versus 100µM furosemide-LLL, 36.8 [24 – 64.8], N = 10-13, $p > 0.05$), suggesting that effects may be epithelial in nature and independent of basolateral Cl^- co-transport. In the organ bath, 5% LLL decreased tension indicative of relaxation (Δweight [g]; LLL, -0.15 [-0.19 – -0.11] versus Krebs 0.01 [0 – 0.02], N = 9-10, $p < 0.05$). After incubation with LLL for 30 min, the Carbachol (100µM)-induced contractile response increased (fold change; LLL, 1.62 [1.3 – 1.9] versus Krebs, 1.15 [1 – 1.2], N = 8-9, $p < 0.05$).

Lactobacillus LB significantly influences both intestinal secretomotor function and motility ex-vivo. These effects combined could contribute to the clinical efficacy of this postbiotic.

Poster #18

Antimicrobial resistance associated with neonatal calves

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Abstract

Antimicrobial resistance (AMR) is a serious threat to animal and human health. Livestock and their housing may act as reservoirs for AMR bacteria. In this study, we aimed to contribute to AMR surveillance within dairy farms by characterising the resistomes associated with neonatal calves.

Ten dairy farms were surveyed for >90 parameters including hygiene practices and antibiotic usage. Both culture-based and culture independent techniques including shotgun metagenomic sequencing was used to characterise the resistomes present in calf faeces. Samples were tested *in vitro* for phenotypic AMR against seven antibiotic classes: penicillins, macrolides, phenicols, aminoglycosides, tetracyclines, synthetic and polymyxins. Resistant bacteria were isolated and underwent both 16S rRNA gene and genome sequencing and multidrug resistance (MDR) testing.

The study revealed high AMR diversity and abundance in calf faeces, with AMR levels varying between farms. Resistance to all antibiotic classes, except the polymyxin colistin, was detected on all farms, resistance against neomycin and trimethoprim being highest (maximum CFU/mL of 9.57E+08 and 2.83E+08 respectively). Of the 84 AMR isolates sequenced, 66 isolates (78.6%) were resistant to 3 or more antibiotics and classified as MDR. MDR resistant Escherichia coli isolated from faeces, was phenotypically resistant to 7 antibiotic classes and harboured 68 resistance genes (ARGs). Shotgun metagenomics revealed high ARG abundances within faecal samples, with transcripts per million values of ARGs reaching 13,085.45. This study reveals that the poor calf and farm management practices may contribute to high AMR levels in calf gastrointestinal tracts.

Poster #19

The effect of micro-aerobic conditions on the PrfA region of *L. monocytogenes*

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Abstract

Listeria monocytogenes is a food-borne pathogen which causes listeriosis. It is an intracellular parasite invading the epithelial cells where it escapes from the vacuole into the host cytoplasm to replicate, using actin-based motility to move within and between cells. The intracellular life cycle is well documented whereas the time spent in the lumen of the intestine is poorly understood. The aim of this study was to investigate the mechanism by which *L. monocytogenes* adapts to the environment of the small intestine prior to invasion. Specifically, to determine if the PrfA regulon, that encodes the virulence factors of *L. monocytogenes*, is switched on by signals within the intestinal lumen. Initially three signals were examined, micro-aerobic (5%v/v oxygen), butyrate, a short chain fatty acid molecule synthesised by bacteria within the gut microbiota and serotonin (5-HT), a key neurotransmitter that modulates brain behaviour. 5-HT is secreted by enterochromaffin cells (EC) into the intestinal lumen where it acts to control gut motility, secretion and vasodilation. L. monocytogenes InlA strains with chromosomal phly::egfp or pactA::egfp transcriptional fusions were grown in MD10 media either aerobically or micro-aerobically with and without 5 mM butyrate or 100 µM 5-HT and Gfp expression monitored. There was significant induction of the phly and pactA expression in micro-aerobic versus aerobic conditions. The addition of 5-HT had no effect while butyrate significantly lowered both hly and actA transcription. These data indicate that the PrfA regulon is responsive to signals likely to be encountered in the small intestine.