Faecal Microbiota Transplantation To Tackle Antimicrobial Resistance in Chronic Liver Disease

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ABSTRACT

The World Health Organisation describes antimicrobial resistance (AMR) as one of the biggest threats to global health, food security, and development.[1] AMR infections cause ~700,000 deaths/year globally, predicted to rise to 10 million, costing $100 trillion by 2050 if no action is taken. Indiscriminate use of antimicrobials has driven the emergence of multidrug-resistant bacteria, resistant to 60% of antimicrobials in some countries.[1] **There is a very real threat that we will face a future in which antibiotics no longer work.** There is an evolving crisis of chronic liver disease (CLD) in the UK with the prevalence and mortality increasing exponentially. End-stage CLD, known as cirrhosis, is the third biggest cause of mortality and loss of working life behind ischaemic heart disease. It is the only major cause of mortality and morbidity, which is on the increase in England [2,3]. Over the last decade, the number of people dying with an underlying cause of liver disease in England rose by 400%.[2,3] One in 10 who die from CLD are in their 40s [2,3].

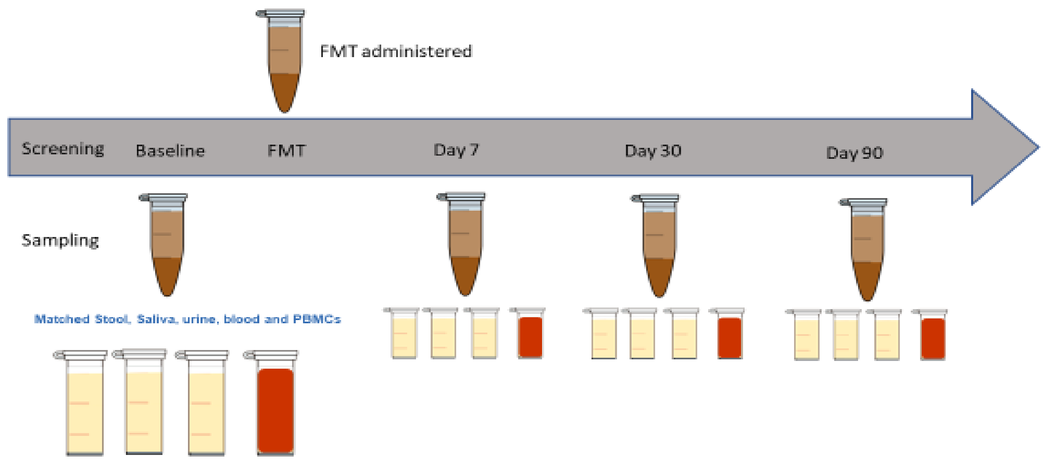
Patients with cirrhosis are at high risk for AMR because they are frequently prescribed antibiotics (~25% are on long-term antibiotics, taking daily quinolones, particularly associated with AMR) as they often develop severe infections, resulting in hospitalisation and potential death. They frequently undergo invasive procedures such as large volume paracentesis and have recurrent hospitalisations[4-6] (decompensated cirrhotics have ~37% 30-day readmission rate).[7] 27% of liver patients at King's College Hospital have infections with multi-drug resistant organisms (MDRO).

The gut microbiome of patients with cirrhosis is 'dysbiotic', characterised by a decrease in diversity, with an overabundance of pathogenic species. The gut microbiome has prime importance in the pathogenesis of cirrhosis with an evolution from health to 'dysbiosis' associated with progression to chronic liver failure.[8] Dysbiosis and bacterial translocation culminate in systemic inflammation, endotoxemia and innate immune dysfunction, referred to as cirrhosis-associated immune dysfunction (CAID) which predisposes to infection resulting in hospitalisation, worsening liver function and associated with high mortality.[9, 10] The gut microbiota are by far the largest reservoir of Multi Drug Resistant Organisms. **We hypothesised that in patients with advanced cirrhosis modifying the gut microbiota could improve clinical outcomes and reduce AMR. As faecal microbiota transplantation (FMT) is an extremely effective treatment for AMR in *C. difficile* infection**

METHODS AND RESULTS

We conducted a prospective, single-centre, randomised, single-blinded, placebo-controlled feasibility trial evaluating faecal microbiota transplantation (FMT) against placebo [11]. Patients with advanced, but stable cirrhosis with a Model for End-stage Liver Disease (MELD) score between 10 and 16 were recruited. We aimed to recruit thirty-two patients from outpatient clinics at King's College Hospital or from suitable inpatients on the wards. The patients were recruited as per the inclusion and exclusion criteria. Patients were randomised in a single-blinded fashion in a ratio of 3:1 FMT to placebo. Patients were unaware of the intervention given, but investigators were not blinded to the treatment intervention. Research Ethics approval was given by the London Southeast Research Ethics committee (ref 17/LO/2081). Trial registration number NCT02862249 and EudraCT 2017-003629-13. In total, 15 patients were randomised to FMT plus standard of care (as per our institutional practice) and six patients to placebo. To assess the stability of the transplanted gut microbiome and its efficacy in modulating the patient's own microbiome, disease and inflammatory status. Blood, saliva, stool and urine samples were collected from participants at baseline and on day 7, 30 and 90 post-FMT administration. FMT/placebo was administered into the jejunum within seven days of baseline [Figure 1].

To enable comparison of the composition of the faecal and oral microbiota with the donor microbiome DNA was extracted from matched stool and saliva samples to be used for metagenomic analyses and for qPCR of selected enteropathogens. Faecal and Plasma Cytokine production and barrier integrity markers were assed by electrochemiluminescence or ELISA[12]. Faecal, blood and urine metabolite profiles were assessed by ^{1}H-Nuclear Magnetic Resonance. Serum samples have been analysed for immune function assays e.g., Tru-culture® to assess the cytokine production after whole blood stimulation with stimulants such as LPS, heat-killed *E. coli* in comparison to null samples.



The mean age of participants was 57.8 years (range 38-75) and the majority were male (76.2%). 20% of patients recruited were colonised with drug resistant bacteria. **Administering FMT to cirrhotic patients significantly reduced stool carriage of *E. faecalis* [*p*=0.000006, Figure 2] and enteropathogenic *E. coli (EPEC)* [*p*=0.0025, Figure 3]** in the FMT treated group but not placebo. EPEC causes contact mediated barrier damage[13] and *E. faecalis* produces a pore-forming toxin cytolysin that causes barrier damage. **FMT did reduce AMR gene carriage [Figure 4]**, particularly reducing the gene MH0233 van ligase. Which gives rise to vancomycin resistance by prevention of binding to *E. faecalis* peptidoglycan.

Reduction in pathogenic species that cause intestinal barrier damage & carry AMR genes

Figure 2: FMT eradicates *Enterococcus faecalis*

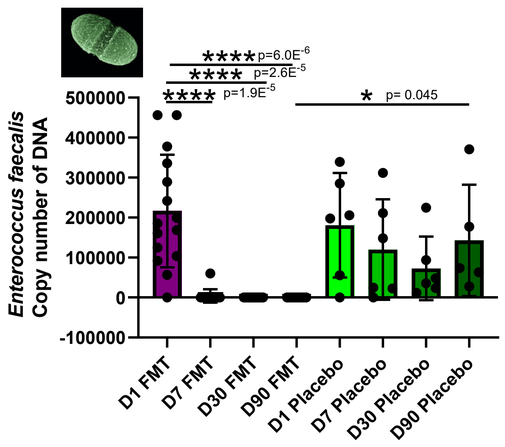
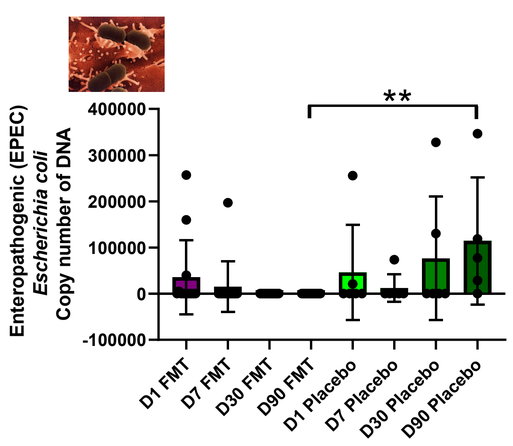


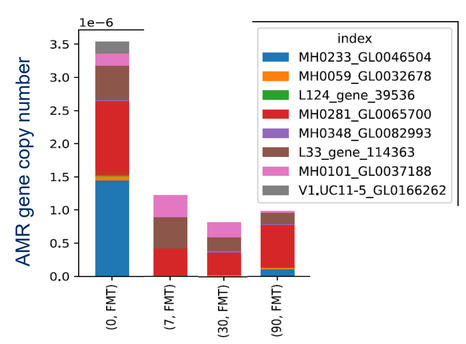
Figure 3: FMT eradicates *Enteropathogenic E. coli*



Intestinal inflammation and barrier damage are known to increase AMR carriage[14] [Figure 7]; Plasma D-Lactate levels decreased at day 7 with a matched increase in faecal D-Lactate after FMT administration. We have previously shown D-Lactate, a marker for barrier damage, is linked to worsening liver cirrhosis[12]; suggesting FMT is inducing barrier repair and a reduction in translocation [Figure 5]. FMT reduced intestinal inflammation including IL-17A production at day 7 post FMT [Figure 6].

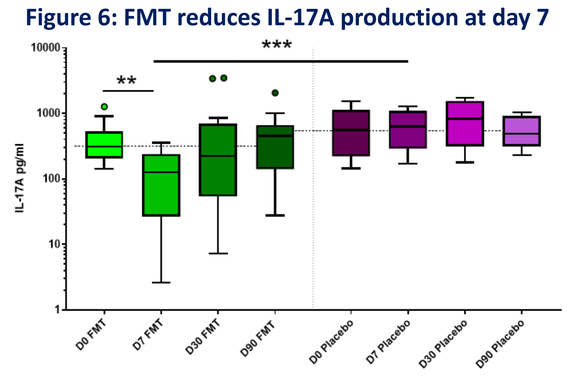
Reduction in AMR genes

Figure 4: FMT reduces AMR gene carriage



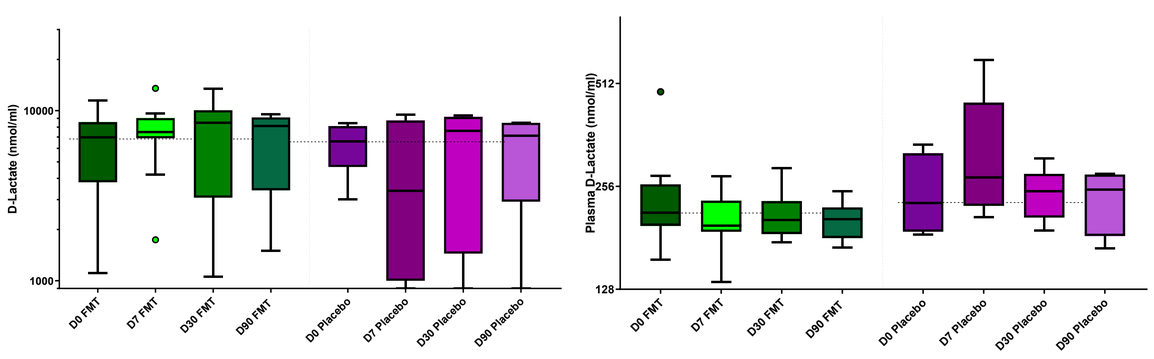
Restoration to mucosal & systemic immunity

Figure 6: FMT reduces IL-17A production at day 7



Improved barrier function

Figure 5: FMT reduces D-Lactate translocation across the gut barrier at day 7



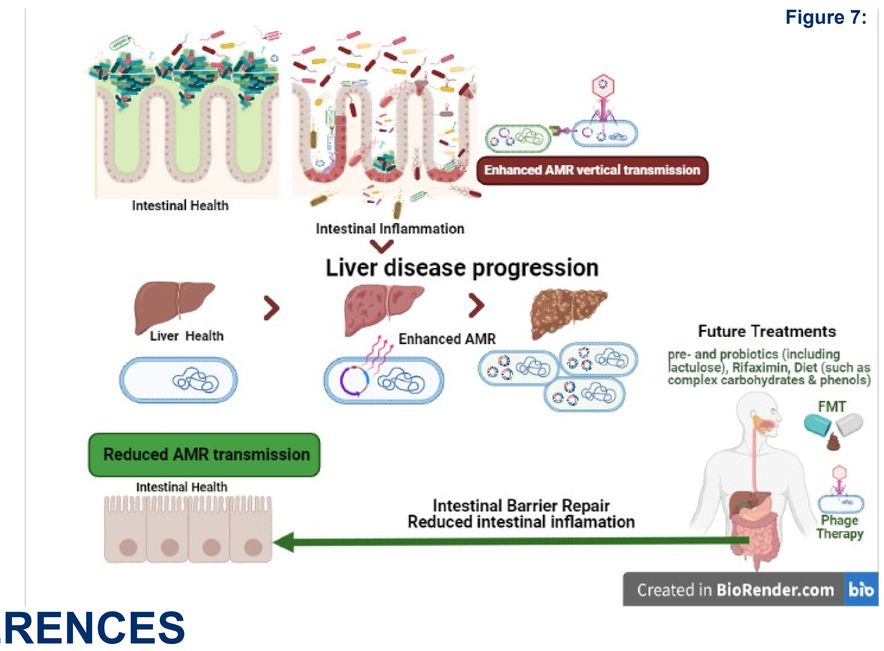
CONCLUSION

Intestinal inflammation and barrier damage have been shown to increase AMR carriage and transmission[14] Bacterial adherence to mucosal surfaces[15] and barrier damage plays a pivotal role in cirrhosis progression[12] making them potential targets in the development of novel antimicrobial therapeutics[13,16] [Figure 7]. Not only can gastrointestinal colonization facilitate horizontal gene transfer, but it can also serve as a reservoir for spread of AMR-organisms into the environment by contamination.

Faecal Microbiota Transplantation (FMT) reduces the pathogenic bacterial burden in cirrhosis, removing enteric pathogens known to cause epithelial barrier disruption. FMT reduced intestinal succinate expression linked to adherence to the mucosa and bacterial pathogenesis. FMT Enhanced butyrate production, which is linked to restoration of intestinal barrier function. FMT also modifies intestinal mucosal and systemic inflammatory profiles in patients with cirrhosis. These data support FMT as playing an important role in inflammatory restoration and altering the gut-microbiota to promote barrier repair and reduce AMR.

Targeting the gut microbiome to prevent and reduce AMR in patients with cirrhosis could make a significant impact on individual patient outcomes as well as reducing environmental contamination with MDROs. The development of therapies that can favorably manipulate the gut microbiome have the potential to have a huge real-world impact on the millions of people who suffer from cirrhosis and whose outcomes are adversely impacted by MDRO infections. This represents a paradigm change in the therapeutic landscape which might conceivably influence clinical guidelines within the decade and lead to greater rationalisation of antibiotics. Such strategies might include faecal microbial transplantation or the use of bacteriophage therapies. Further studies are now needed to prove the efficacy of FMT in larger populations of patients with cirrhosis and to evaluate its mechanisms of action, which we are in the process of doing [NIHR EME PROMISE Trial].

Figure 7:



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