**APPLICATION TO THE QUEEN ELIZABETH HOSPITAL RESEARCH ETHICS COMMITTEE**

**1. TITLE**

Gastrointestinal eradication of resistant Gram negative bacteria by faecal microbiota transplantation (FMT)

**2. INVESTIGATORS DETAILS AND QUALIFICATION**

Dr Lito Papanicolas (MBBS, FRACP, FRCPA)

Infectious Diseases Physician

The Royal Adelaide Hospital

Adelaide SA 5000

Tel. 0449780613

Email: lito.papanicolas@sa.gov.au

PhD student – Flinders University and SA Health and Medical Research Institute

Dr Samuel Costello (MBBS, FRACP)

Gastroenterologist

Queen Elizabeth Hospital

Woodville Road

Woodville SA 5011

Tel. 0413311793

Email: sam.costello@sa.gov.au

Dr Morgyn Warner (MBBS, FRACP, FRCPA PhD)

Infectious Disease Physician and Microbiologist

Queen Elizabeth Hospital

Woodville Road

Woodville SA 5011

Tel.

Email: morgyn.warner@sa.gov.au

Dr Renjy Nelson (MBBS, FRACP)

Infectious Disease Physician

Queen Elizabeth Hospital

Woodville Road

Woodville SA 5011

Tel. 0431414951

Email: renjy.nelson@sa.gov.au

Dr Robert Bryant (MBBS, FRACP, MScR)

Gastroenterologist

Queen Elizabeth Hospital

Woodville Road

Woodville SA 5011

Tel. 0448447795

Email: Robert.Bryant@sa.gov.au

Dr Robert Carroll (MBBS, FRACP

Renal Physician

Royal Adelaide Hospital

Adelaide SA 5000

Email: Robert.carroll@sa.gov.au

**3. PURPOSE OF STUDY**

**Objectives of study**

**Primary**

* To determine whether faecal microbiota transplantation (FMT) can decrease carriage of selected resistant Gram negative bacteria (RGNB) from the gastrointestinal tract, as evaluated by a significant decrease in relative abundance of these organisms in the stool microbial community.

**Secondary**

* To evaluate whether FMT diminishes subsequent rates of clinical infection in persons with recurrent Gram negative bacterial infections.
* To evaluate the effect of FMT on bacterial resistance genes in the recipient microbiota
* To evaluate changes in the ability to culture resistant Gram negatives from stool after FMT
* To evaluate patient perception and experience of FMT

**4. BACKGROUND**

The world is facing a global epidemic of antimicrobial resistance which poses a grave threat to human health as antibiotics lose their efficacy ([1](#_ENREF_1)). The major groups of multi-resistant organisms (MROs) which are of most concern in our hospitals include methicillin resistant Staphylococcus aureus (MRSA), Vancomycin resistant Enterococci (VRE), extended-spectrum beta-lactamase producing organisms (ESBLs) and carbapenem resistant Enterobacteriaceae (CRE). Unlike MRSA or VRE, which refer to specific gram positive organisms, ESBL-type resistance is a broad term that encompasses hundreds of individual resistance genes, which are transmissible between similar gram negative organisms ([2](#_ENREF_2)). ESBLs and the Amp-C genes, which give slightly different resistance profiles to other ESBLs, are often grouped together for infection control purposes. The carbapenemases which give rise to a carbapenem resistant phenotype are also produced by a very diverse, albeit less numerous, group of resistance genes ([2](#_ENREF_2)).

Testing for these MROs at SA Pathology is based on culture of colonised sites- the nares in the case of MRSA and stool or rectal swabs for the other organisms. Bacterial isolates that grow on selective media can then be identified and tested for particular resistance genes that are known to be transmissible (ie not intrinsic to that organism) by polymerase chain reaction (PCR).

Current infection control efforts, including personal protective equipment, hand hygiene and decolonisation to the skin surfaces, have been most effective in decreasing the incidence of MRSA, a skin colonizer ([3](#_ENREF_3)), resulting in a decreasing incidence of MRSA in developed countries. However, the MROs which colonise the gut have proven more difficult to control and there is a world-wide trend of increasing incidence of these organisms ([4](#_ENREF_4), [5](#_ENREF_5)).

Of particular concern are the resistant gram-negative enteric bacteria such as *E. coli* and *Klebsiella pneumoniae* of the Enterobacteriaceae family which can cause severe infection in otherwise healthy people. These infections have emerged in the community and are not limited to the hospital setting, manifest predominantly as urinary tract infections in otherwise healthy people ([4](#_ENREF_4)). This is in contrast to VRE infections, which tend to affect chronically ill hospitalised patients. The most worrying MROs are the CREs carrying transmissible carbapenem resistance genes. These organisms are intrinsically virulent, and carry resistance not only to carbapenems but in some cases to almost every other known antibiotic ([5](#_ENREF_5)). Cases are extremely difficult to treat, and are associated with a high mortality and significant health care costs([1](#_ENREF_1)). CRE are already endemic in some regions of the world including Southern Europe, the Middle East and South Asia and is rapidly increasing in incidence ([5](#_ENREF_5)). Although rarely encountered in Australia, small outbreaks have already occurred interstate, and the global trend would predict that local outbreaks are imminent. It is thus imperative that innovative approaches are developed to reduce the spread of these organisms.

When intact, the human gut microbiome has the ability to prevent colonisation with MROs, a property known as ‘colonisation resistance’ ([19](#_ENREF_19)). However, when the microbiome is disrupted by antibiotic use, subsequent colonisation with resistant pathogens can occur. In mouse models it is the reduction of microbial diversity resultant from antibiotic administration that allows establishment of VRE or multi-resistant *Klebsiella.* In allogeneic hematopoietic stem cell transplant patients the reduction of microbial diversity that occurs during treatment often results in the dominance of a single microbial taxon in the microbiome. This in turn is a predictor of subsequent bacteraemia with a 9-fold increased risk the case of VRE dominance, and a 5-fold increased risk in the case of gram-negative Proteobacteria dominance ([20](#_ENREF_20)). These observations form the basis of the theory that restoration of the microbiome with FMT following disruption may prevent MRO colonisation, and thereby prevent serious clinical infections resulting from these dominant pathogenic bacteria.

FMT was first reported in humans by Eiseman et al in 1958 in the treatment of 4 patients with pseudomembranous colitis ([6](#_ENREF_6)). Three of the four patients were described as terminally or critically ill requiring vasopressor support and all were successfully cured. Over the subsequent years there have been case reports and case series describing FMT predominantly for *Clostridium difficile* colitis but also for treating IBD, irritable bowel syndrome and constipation ([7-10](#_ENREF_7)). In the past decade, there has been a heightened interest in the use of this therapy, predominantly driven by increasing rates of recurrent *C. difficile* infection. During this time *C. difficile* has become more frequent, more severe and more refractory to standard treatment as well as more likely to relapse ([11](#_ENREF_11)). Standard treatment with metronidazole or vancomycin alters the normal gut flora, resulting in decreased microbial diversity that would usually provide colonization resistance against *C. difficile* infection. For this reason, after successful initial therapy, up to 35% of patients will experience a symptomatic recurrence after ceasing antibiotics ([12](#_ENREF_12)). A subset of patients will have multiple recurrences and subsequent relapses occur in 45-65% of patients who have relapsed one or more times ([13](#_ENREF_13)). For patients with recurrent *C. difficile* colitis, FMT offers the greatest chance of cure of any therapy with success in 81 and 94% ([14-18](#_ENREF_14)). This impressive success rate is presumably due to the ability of the transplanted bacteria to recolonize or occupy the missing components and niches of the normal intestinal microbiota, thus removing the microbial niche that *C. difficile* would otherwise exploit.

There is evidence from a study in mice that VRE and Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) colonized mice can be decolonised with FMT from healthy animals ([25](#_ENREF_25)). In this study, mice were treated with ampicillin administered via drinking water and then inoculated with either CRKP or VRE. Mice were then randomised to receive FMT from the stool of a healthy mouse or phosphate buffered saline. Following FMT treatment, *K. pneumoniae* density in faecal pellets decreased within one day and became undetectable within 7 days in all mice. VRE, on the other hand, was cleared in 60% of the mice but reduced by 3 logs in the remaining 40%.

FMT explicitly for the treatment of multi-resistant organisms has been reported in the literature once in single case described by Singh *et al*. (21). This case describes a 60 year old man who suffered recurrent infection of his transplanted kidney allograft with ESBL producing *E. coli*. FMT was administered via nasoduodenal tube and this resulted in clearance of the organism after 1 week. He remained clear of both clinical infections and faecal colonisation for 12 weeks ([21](#_ENREF_21)). Three other cases exist where patients colonised with an MRO received FMT for treatment of *C. difficile* colitis. The first case describes a woman in her 30s who was a recipient of kidney and cardiac transplants. Prior to the FMT she had significant clinical infection with VRE including urinary tract infections and bacteraemia. She received FMT via nasogastric tube. Analysis of the patient’s faecal microbiota following the transplant showed a relative reduction of the abundance of *C.difficile* and VRE and the microbiome and she experienced no further clinical infections with VRE ([22](#_ENREF_22)). In the second case, a patient colonised with VRE was clinically recovered from *C. difficile* colitis post FMT but VRE continued to be isolated from the patient’s stool for 3 months post transplant ([23](#_ENREF_23)). In the final case, a 66 year old man received FMT for *C. difficile*. Prior to this he had been colonised with 12 MROs including CRE and VRE due to chronic debilitation and long-term residence in the intensive care. Following FMT, the majority of MROs were eradicated, including the CRE and the VRE, and the patient had a marked reduction in clinical episodes of infection ([24](#_ENREF_24)).

There is also emerging evidence that FMT may reduce recurrent urinary tract infection. Tariq et al (26) reported that FMT performed for *C. difficile* infection also significantly decreased the frequency of recurrent UTI in these patients and improved the antibiotic susceptibility profile of the organisms causing UTI. The likely mechanism is that dysbiosis from previous antibiotic therapy has reduced the colonisation resistance provided by a normal gut microbiome and allowed pathogens that cause UTI to become established in the gut (26). Wang et al also reported a case in which FMT interrupted a 25 year history of recurrent urinary tract infection (27).

Thus, there is burgeoning evidence to suggest that FMT may increase faecal microbial diversity and therebyreduce carriage of pathogens with antimicrobial resistance, and reduce subsequent infections with these pathogens. Available human data is limited to case-reports, and there is a pressing need to evaluate the efficacy of FMT for elimination of resistant bacteria in a controlled trial given the global threat posed by these organisms.

**Standard of care for patients colonized with resistant Gram negative bacteria.**

Currently there is no routinely available decolonization therapy or procedure available to eliminate gut carriage of resistant Gram negative organisms. People may naturally become clear of these organisms over a period of months to years, however clearance rates are highly variable between individuals ([8](#_ENREF_26)). Clinical infections with these organisms are treated with appropriate targeted antibiotic therapy, usually upon advice of an infectious diseases physician.

**5. ETHICAL CONSIDERATIONS**

**Benefits of the study**

The purpose of this study is to investigate whether FMT reduces and eliminates carriage of MROs in colonised individuals, and thereby diminishes rates of spread and clinical infection with these organisms. The potential benefit of the study is significant, given that the global incidence of MROs is rapidly rising, and that there are no current effective strategies for therapy. The FMT study may therefore be of benefit to not only the participants, but to the community at large.

**Risks of the study**

There have been no reports of major complication of faecal transplant in the literature to date other than complications of sedation in those patients receiving FMT via endoscopy or colonoscopy. However, FMT has only been performed in large numbers in the past decade, and there may be yet unknown long term risks of FMT.

It is not anticipated that the faecal transplant procedure will cause any adverse reactions, but participants will be provided with information about supports they can contact should they experience any distress in relation to the study.

Participation in the study will not alter routine clinical care for those enrolled. All treatment decisions remain at the discretion of the usual treating physician,

**Consent, confidentiality, data storage, security, and publication.**

Before taking part in the study, informed written consent will be obtained from patients. The researchers will ensure that the patient is given full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the trial. They will be given sufficient time to consider the information, to ask questions and to seek advice prior to being asked whether they wish to participate in the study. Participants will also be assured their participation in the trial is voluntary. The participation is strictly confidential, and the identity of subjects will not be disclosed to other medical or research staff unless subjects agree.

Once subjects have been enrolled in this study, they will be given a study participant code, and only study investigators will have access to their name and personal details. The data will be stored on the Central Adelaide Local Health Network (CALHN) central server in a password protected encrypted file. The data will be stored as such for 5 years beyond study completion, following which, the lead investigator will dispose of the study data via electronic deletion.

We intend to summarise the results in a manuscript and to submit it for publication in a peer-reviewed journal. Therefore all information gathered from this study will be published in a form that does not allow patient identification.

Our proposed study has the support of the heads of infectious diseases and gastroenterology at the Queen Elizabeth Hospital and Royal Adelaide Hospital as well as the head of microbiome research at SAHMRI.

**Sample storage**

Within 1 hour of defecation, faecal samples will be transported on ice to the SAHMRI for frozen storage prior to analysis. They will be stored in sealed specimen containers within sealed and labelled (de-identified, re-identifiable) bags. Following analysis for microbiota composition and metabolites, the faecal samples will be degraded and will be discarded at the SAHMRI according to safe biohazard protocols.

**Conflicts of interests**

The lead and associate investigators may be the primary clinicians responsible for the management of patients within the study cohort. This poses the only conceivable theoretical conflict of interest for the proposed study, in the setting of an unequal relationship between participants and investigators. The investigators have mitigated this conflict of interest by detailing in the patient information sheet:

* “Participation in this study is voluntary and if you choose to opt out, this will not compromise your routine clinical care nor your involvement in the existing study protocol.”
	+ “You may also withdraw from the study at any time and for any reason or no reason
* Personal or other sensitive information beyond that accessible during the process of routine clinical care is not required for the study.
* The investigators have no affiliation or involvement in any organisation or entity with direct or indirect interest in the subject matter of this research.

**6. SUBJECTS**

***Inclusion criteria:***

* + Age range 18-85 years inclusive.
	+ Patients with refractory or recurrent infection–defined as at least 3 episodes of infection requiring at least 3 episodes of antibiotic therapy in preceding 12 months.
		- Infection caused by an Gram negative bacterium which has acquired resistance to clinically important antibiotics routinely reported by SA Pathology. This is defined as reported resistance to one or more of the following antibiotics**: meropenem, ceftriaxone, cefepime, ceftazidime, cefalexin, amoxicillin-clavulanate, piperacillin-tazobactam, ciprofloxacin, gentamicin or trimethoprim**

*Exclusion criteria:*

* Active gastrointestinal infection
	+ Ie bacterial or viral infection causing symptoms of diarrhoea
	+ colonisation with RGNB is not considered to be an infection
* Pregnancy
* Current use of antibiotics\*
* Cognitive impairment
* Perianal inflammation
* Life expectancy < 1 year
* Neutropaenia <0.5 X109/L
* Severe IgE mediated food allergy: urticaria or anaphylaxis
* At risk of peritonitis: including patients with ascites or peritoneal dialysis

**\*** The use of trimethropim-sulfamethoxazole for pneumocystis prophylaxis in immunocompromised patients is excepted.

**7. STUDY DESIGN**

We will enrol 22 patients with infection with recurrent RGNB infection. 11 patients will be randomised to receive faecal transplant and 11 will be randomised to receive placebo (own stool).

**FMT recipient work-up**

* + Patient questionnaire regarding perception and expectation of faecal transplant prior to procedure and at the end of the study.
	+ Detailed past medical history
		- Number and type of infections due to the RGNB
		- Duration of colonisation
		- Medication history (current and prior)
		- Surgical history
		- Hospitalisation
		- Comorbid disease
	+ Stool infection screen for VRE, ESBL, AMP-C and CRE. MC+S, *C. difficile* toxin, Ova, cysts and parasites.
	+ Faecal associated microbiota analysis and culture of stool 1 week prior to FMT.

**FMT donor work-up**

**Medical interview (exclusions)**

* Age: <18 or >65
* Antimicrobial therapy or probiotics in the past 3 months
* Active medical illness or symptoms
* Any medications (other than oral contraceptive pill)
* International travel in last 6 months to areas at high risk of travellers’ diarrhoea
* High risk sexual activity (unprotected sex in last 1 month outside of a monogamous relationship, men who have sex with men, sex for drugs or money)
* Illicit drug use
* Tattoo or body piercing within 6 months
* Known HIV or viral hepatitis exposure in the last 12 months
* Incarceration or a history of incarceration.
* Family history of colorectal carcinoma involving 2 or more first degree relatives
* Household members with active GI infection

**Medical history and Examination (exclusions)**

* Any gastrointestinal disorder
* Obesity (BMI>30),hypertension, type 2 diabetes and dyslipidaemia
* Malnutrition (BMI <18)
* Autoimmune disease
* Atopic disease
* Depression
* Infection with HIV, Syphilis, Hepatitis B or C
* Malignancy
* Chronic pain syndromes, neurologic or neurodevelopmental disorders

**Blood screening**

* Full blood count (Anaemia, WCC>12.5 are exclusions)
* Electrolytes, Urea and Creatinine (renal impairment eGFR<60)
* Liver function tests (abnormal LFTs are exclusions)
* Human T-cell lymphotropic virus 1 and 2 serology
* Epstein Barr Virus IgM and IgG
* Cytomegalovirus IgM and IgG
* Syphilis (Rapid plasma reagin)
* *Strongyloides stercoralis*, *Entamoeba histolytica*, *Helicobacter pylori*,
* Toxoplasma serology
* Hepatitis A virus IgM
* Hepatitis B surface Antigen, core Antibody
* Hepatitis C PCR
* HIV PCR
* Antinuclear antibody(ANA)\*
* Fasting lipids and Blood sugar level
* C-Reactive Protein

**\*A positive ANA will not result in exclusion in the following circumstances:**

1**.** All patients with a dense fine speckled (DFS) pattern of ANA may be included as donors – as this pattern is not associated with autoimmune disease or other adverse outcomes.

2. Women with ANA titre ≤1/160 homogenous could be included as donors.

3. Men with ANA titre ≤1/160 homogenous or speckled pattern, or women with ≤1/160​ speckled pattern could be included as donors if they have also tested negative for ENA and ds DNA.

Patients with other patterns or higher titre ANAs will be excluded.

**Stool screening**

* Microscopy and Culture
* Rotavirus, Norovirus and Adenovirus PCR
* *Clostridium difficle* toxin PCR
* Egg, cysts and parasites (including Cryptosporidium spp., Giardia spp., and *Entamoeba histolytica* PCR)
* Vancomycin resistant enterococcus screen, ESBLs, AMP-C and CRE
* Rectal swab testing for chlamydia, gonorrhea and herpes simplex virus DNA

Donors will be anonymous volunteers. They will be paid $18 per hour for time spent participating in the study. If donors are excluded from the study because of an abnormality detected on their screening test, they will be referred to their GP for follow-up. Alternatively, if an STI is detected they could elect to attend clinic 275 for confidential follow-up.

Stool (25%) will be blended with normal saline (65%) and glycerol (10%) in a 5:13:2 ratio and then divided into 200mLaliquots prior to freezing. Each stool aliquot will then be numbered and recorded in the secure and confidential faecal transplant aliquot document that will list the stool donor who provided each aliquot. In this way, any possible transmission of infection or other disease could be traced. A small amount of each individual donation will be set aside and frozen individually. This will allow repeat testing and tracing of each individual donation in the future in the event of possible transmission of infection.

Before transplantation into the study participants several donor stools will be pooled to create as uniform a specimen as possible for each participant. Stool from a minimum of 3 and maximum of 10 donors will be pooled. Pooled samples will be tested for common bacterial and viral pathogens prior to being used. Aliquots of 67 mL of the pooled donation will be frozen and thawed just before transplantation into the participant. The total FMT delivered will be 200 mL over 3 enemas, equivalent to 50 grams in stool content.

Each subject potentially suitable for the study, will also be asked to donate a stool sample of their own. A small portion of the stool will undergo faecal associated microbiota analysis.

Donors will be anonymous and so will not be known to the recipient. This avoids any apportion of blame towards a known donor should a complication or treatment failure arise during the trial.

Enema will be used to deliver the FMT. This is an invasive procedure however it carries a very low risk. There are many medications that are delivered via enema without complications. Prior to receiving the enema, subjects will be offered 2mg loperamide tablet to help with retention of the enema. This would be expected to cause reduction in bowel motions for 24 hours.

**Documentation and tracing of donors**

Each stool donor will be recorded in the study “stool donor register” document. This will include the donor’s name, date of birth, address and contact details. It will also record the result of screening history, physical examination and blood and stool tests. Each donor will be assigned a donor number.

After collection each donation will be screened for known bacterial pathogens (and each donor will also be screened as outlined in the protocol for other illnesses), then a small amount of each individual donation will be set aside, labelled with a name and donor number, and frozen individually. This will allow repeat testing for some pathogens and tracing of each individual donation in the future in the event of possible transmission of infection.

Each stool aliquot will then be numbered and recorded in a secure and confidential document designated the “faecal transplant aliquot document” (to be viewed only by the study investigators). This will list the stool donor number that contributed to each aliquot. In this way any possible transmission of infection could be traced.

**Study Design**

This is a randomised controlled and double-blinded pilot study.

Patients identified as having recurrent infection with resistant Gram negative pathogens will be offered FMT with the aim of clearing the organism/gene from faeces, and preventing recurrent clinical infection with these organisms. After enrollment subjects will provide a stool sample to determine if they are still carrying resistant bacteria and therefore whether they are still eligible for the study. Following confirmation of eligibility, patients will be randomly allocated into either the FMT or control group. Patients will be blinded regarding group allocation.

The 10 subjects in the faecal transplant group will undergo FMT via 3 enemas given 1 week apart. Stool will be collected from study subjects via swabs weekly for 4 weeks, then monthly for one year. These swabs will be stored in a portable freezer. Stool will be analysed for the presence of the resistance genes as well as microbial diversity.

The 10 subjects in the control group will undergo identical enema procedures but will receive their own stool as an enema, prepared from the stool sample provided at enrollment. They will have stool samples collected at the same intervals as the treatment group.

The pooled FMT donor and auto-transplants will be prepared by Dr. Papanicolas. Subject allocation will be performed by a CALHN clinical trial nurse affiliated with the Department of Gastroenterology or Infectious Diseases. Gastroenterologists Dr. Costello, Dr. Bryant or a Gastroenterology registrar will perform the faecal transplantation enema procedure. The proceduralist who will administer the faecal enemas will be not be blinded as to which group the participant has been assigned to, but these investigators will not be involved in any aspect of participant follow-up or data analysis until the study has been completed. This is so the proceduralist can provide a bedside check to ensure the participant does not receive an incorrect fecal transplant – an event which would have serious safety implications for the participant.

Patients will be interviewed monthly by telephone to determine whether they have developed any new clinical infections. Antibiotic and healthcare use for any other reason will also be recorded.

**Care during the follow up period**

During the trial subjects will be treated as per the standard of care for any medical conditions that they have. Patients will be given the telephone number of the primary investigator to contact should they have any concerns. Patients will not be taking antibiotics at enrolment, however, if the need arises for antibiotics during the study period there will be no restriction on the use of antibiotics but their use will be noted in the study notes. Potential donors who are screen failures due to detection of medical conditions will be referred for appropriate care.

**Outcome measures**

* + Primary outcomes:
		- Significant reduction of Gram negative pathogens in the stool as determined by the changes in relative abundance of these organisms in the stool microbiota.
		- Reduction in clinical infections due to the multi-resistant organism during the one year follow-up period.
	+ Secondary outcomes
		- Changes in antimicrobial gene resistance profile in the stool microbiota
		- Increased microbial diversity following FMT
		- Differences in faecal associated microbiota between donor and recipient
		- Patient perception and experience with FMT

**Participation**

Participants will be recruited from:

The Queen Elizabeth Hospital (TQEH) & Royal Adelaide Hospital (RAH) by microbiologists and ID physicians.

All patients will be contacted via their treating clinician, after their treating physician has been consulted, in whichever way the clinician feels is most appropriate to the particular patient.

Demographic details of non-responders will be recorded to enable a full description of the sources of possible bias. All who agree to participate will be subsequently screened to ensure they fulfill inclusion criteria.

Donors will be recruited with a flier advertisement on notice boards at the RAH and TQEH as well as the Adelaide University Medical School, Adelaide University and Flinders University campus. There is an established donor pool through an existing study with CALHN ethical approval (HREC number 121218)

**Withdrawal criteria**

Patients may withdraw from the study at any time. We will ask for their

reasons for statistical purposes, however they will not be obliged to provide

this. Withdrawal from the study will not affect ongoing standard medical care

in any way. Their clinicians will be informed of their participation in the study.

We will ask patients to notify us of any changes in their treatment during the course of the study and if necessary, we will seek their permission to verify this with their treating

clinician.

**8. Specific safety considerations**

There have been two deaths directly attributable to FMT with both of these patients developing aspiration pneumonia. The first patient aspirated during sedated endoscopic FMT delivery to the duodenum ([29](#_ENREF_27)) and the other aspirated during the anaesthetic for colonoscopic FMT ([30](#_ENREF_28)). One other death following FMT occurred due to toxic megacolon and sepsis (31) however this may have been attributable to the recurrence of the underlying *C difficile* infection or a gastrostomy tube leak and not the FMT([32](#_ENREF_30)). There have been no other directly attributable long-term side effects of FMT in over 600 cases in the literature([33](#_ENREF_31)), and many other unreported cases. In a follow up study of 77 patients who had received FMT for recurrent CDI, 4 patients developed new autoimmune disease during the follow up period of 3-68 months ([34](#_ENREF_32)). This study had no control group with which to compare the follow up data and so no association between FMT and the development of autoimmune disease could be made. There is however, a paucity of long-term data and so the possibility of as yet unknown long-term risk needs to be factored into any screening protocol and discussed with patients when consenting patients for FMT.

The risk of infection transmission is minimized with thorough screening. Obesity and insulin resistance are common in the community and the transmission of the metabolic phenotype is a potential risk. Transmission of an obese phenotype has been demonstrated in animal studies([5](#_ENREF_33)) and the possible transmission of obesity been reported in a single human case report ([6](#_ENREF_34))(although there were a number of other factors that may have explained the weight gain seen in this patient). Increased insulin sensitivity has been demonstrated in obese subjects following duodenal infusion of faeces from lean donors ([37](#_ENREF_35)). The transmission of insulin resistance via FMT is therefore a potential risk. It is for this reason that we suggest obesity, type 2 diabetes mellitus, hypertension and dyslipidaemia should be screened for on history, examination and blood testing. Furthermore, the development of metabolic syndrome, autoimmune disease, or any other new illness will be screened for during the 12 month follow-up period. Our stool screening protocol was adapted from previous guidelines([38](#_ENREF_36), [39](#_ENREF_37)) with the addition of the metabolic syndrome screen, *Strongyloides stercoralis*, *Entamoeba histolytica* serology, PCR testing for stool parasites as well as stool multi-resistant organism screen .

**9. Analysis and reporting of results**

All of the outlined techniques are well established and are in keeping with international practice. In addition, the proposed FMT methods are in current use for a trial of FMT in ulcerative colitis within CALHN (HREC number121218).

Faecal associated microbiota analysis will be undertaken 1 week prior to FMT and daily in the first week following FMT and then twice weekly for the subsequent 3 weeks. Analysis of microbiota composition, resistance genes and microbiota metabolites will be performed using molecular methods. Ribosomal RNA 16S sequencing analysis will be performed using the Illumina MiSeq platform and depending on changes seen in the microbiota, further metagenomics sequencing will be undertaken. Dr Geraint Rogers (SAHMRI) will perform the microbiota sequencing. Dr Rogers has extensive experience with 16S and metagenomics sequencing analysis and the necessary facilities and support to perform the analysis.

**Statistical analysis**

Patient information will be de-identified and the results of microbiota analysis and MRO colonisation will be recorded in an excel spread sheet. This data will then be imported into the SPSS program for statistical analysis. We have access to statistical analysis from the University of Adelaide department of statistics.

The sample size for the study was calculated using a binary outcome superiority trial power calculation. From animal study data (25) we expect at least an approximately 80% success in the intervention group and a 20% elimination rate in the placebo group. Significance level (alpha) was 0.05 with a power of 90%. This yielded a sample size required per group of 10 with a total sample size of 20.

Sample size was also calculated for the second primary outcome of reduction in recurrent infection rate. For patients who have 3 or more recurrent infections in the preceeding year the power required to show a 50% reduction in recurrent infection frequency is 21 (80% power at 0.05 significance).

**Budget**

Screening costs are currently $650 per potential donor and we expect that we would need to screen approximately 6-8 potential donors to have 2 donors pass screening. This would be a total cost of $5,200 for screening.

Phenotypic screening of stool samples on selective agar: ~$12/sample (includes agar plates and storage tubes) X 310 samples = 3,720

Resistance gene PCR testing = ~$5,280

Stationary for participant diaries = $100

TGA clinical trials registration fee =$330

Biostatistician= $500

Total costs: $15,000

Illumina MiSeq 16s RNA genomic sequencing costs will be covered by SAHMRI

We will apply for grants from the hospital research funds of both the Royal Adelaide and Queen Elizabeth Hospitals. We will also apply to the Infectious Diseases society of Australia research fund as well as a number of benevolent organisations that sponsor medical research. **References**

1. Organisation WH. Antimicrobial Resistance Global Report on Surveillance. 2014.

2. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 2010;54:969-76.

3. Derde LP, Cooper BS, Goossens H, et al. Interventions to reduce colonisation and transmission of antimicrobial-resistant bacteria in intensive care units: an interrupted time series study and cluster randomised trial. Lancet Infect Dis 2014;14:31-9.

4. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 2008;8:159-66.

5. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011;17:1791-8.

6. Eiseman B, Silen W, Bascom GS, et al. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery 1958;44:854-9.

7. Landy J, Al-Hassi HO, McLaughlin SD, et al. Review article: faecal transplantation therapy for gastrointestinal disease. Aliment Pharmacol Ther 2011;34:409-15.

8. Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol 2012;9:88-96.

9. Borody TJ, Warren EF, Leis S, et al. Treatment of ulcerative colitis using fecal bacteriotherapy. J Clin Gastroenterol 2003;37:42-7.

10. Moayyedi P, Surette M, Kim P, et al. Fecal Microbiota Transplantation Induces Remission in Patients with Active Ulcerative Colitis in a Randomized Contolled Trial. Gastroenterology 2015;149:102-109.

11. Kelly CP, LaMont JT. Clostridium difficile--more difficult than ever. N Engl J Med 2008;359:1932-40.

12. Pepin J. Improving the treatment of Clostridium difficile-associated disease: where should we start? Clin Infect Dis 2006;43:553-5.

13. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. Am J Gastroenterol 2002;97:1769-75.

14. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis 2011;53:994-1002.

15. Hamilton MJ, Weingarden AR, Sadowsky MJ, et al. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol 2012;107:761-7.

16. Costello SP, Conlon MA, Vuaran MS, et al. Faecal microbiota transplant for recurrent Clostridium difficile infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. Aliment Pharmacol Ther 2015;42:1011-8.

17. Kassam Z, Lee CH, Yuan Y, et al. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500-8.

18. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013;368:407-15.

19. Tosh PK, McDonald LC. Infection control in the multidrug-resistant era: tending the human microbiome. Clin Infect Dis 2012;54:707-13.

20. Taur Y, Xavier JB, Lipuma L, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 2012;55:905-14.

21. Singh R, van Nood E, Nieuwdorp M, et al. Donor feces infusion for eradication of Extended Spectrum beta-Lactamase producing Escherichia coli in a patient with end stage renal disease. Clin Microbiol Infect 2014;20:O977-8.

22. Stripling J, Kumar R, Baddley JW, et al. Loss of Vancomycin-Resistant Enterococcus Fecal Dominance in an Organ Transplant Patient With Clostridium difficile Colitis After Fecal Microbiota Transplant. Open Forum Infect Dis 2015;2:ofv078.

23. Jang MO, An JH, Jung SI, et al. Refractory Clostridium difficile Infection Cured With Fecal Microbiota Transplantation in Vancomycin-Resistant Enterococcus Colonized Patient. Intest Res 2015;13:80-4.

24. Crum-Cianflone NF, Sullivan E, Ballon-Landa G. Fecal microbiota transplantation and successful resolution of multidrug-resistant-organism colonization. J Clin Microbiol 2015;53:1986-9.

25. Caballero S, Carter R, Ke X, et al. Distinct but Spatially Overlapping Intestinal Niches for Vancomycin-Resistant Enterococcus faecium and Carbapenem-Resistant Klebsiella pneumoniae. PLoS Pathog 2015;11:e1005132.

26. Tariq, R et al. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection Reduces Recurrent Urinary Tract Infection Frequency. Clin Infect Dis 2017;65:1745-1747.

27. Wang T et al. Fecal Microbiota Transplant for Refractory Clostridium difficile Infection Interrupts 25-Year History of Recurrent Urinary Tract Infections. Open Forum Infect Dis 2018;5(2). 28. A F, S E. Use of stool transplantation to clear fecal colonization with carbapenem-resistant Enterobacteriaceae (CRE): proof of concept, abstr 1805. Abstr Soc Healthcare Epidemiol AM (SHEA) IDWeek 2014, 11 October 2014, Philadelphia, PA. 2014.

29. Baxter M, Ahmad T, Colville A, et al. Fatal Aspiration Pneumonia as a Complication of Fecal Microbiota Transplant. Clin Infect Dis 2015;61:136-7.

30. Kelly CR, Ihunnah C, Fischer M, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. Am J Gastroenterol 31. Solari PR, Fairchild PG, Noa LJ, et al. Tempered enthusiasm for fecal transplant. Clin Infect Dis 2014;59:319.

32. Moore TA, Rodriguez A, Bakken JS. Reply to Solari et al. Clin Infect Dis 2014;59:319-20.

33. Kassam Z, Lee CH, Yuan Y, et al. Navigating long-term safety in fecal microbiota transplantation. Am J Gastroenterol 2013;108:1538.

34. Brandt LJ, Aroniadis OC, Mellow M, et al. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. Am J Gastroenterol 2012;107:1079-87.

35. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:1241214.

36. Alang N KC. Weight gain after fecal microbiota transplantation. Open Forum Infect Dis 2015;2.

37. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143:913-6 e7.

38. Bakken JS, Borody T, Brandt LJ, et al. Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol 2011;9:1044-9.

39. Mattila E, Uusitalo-Seppala R, Wuorela M, et al. Fecal transplantation, through colonoscopy, is effective therapy for recurrent Clostridium difficile infection. Gastroenterology 2012;142:490-6.

**Other ethics committees to which submitted**

Under the SA Health mutual recognition scheme, ethics approval will not be sought at other South Australian hospitals.

**Date of proposed commencement**

*January 2016, once approved.*

**Date of proposed completion**

*June 2017*

**Resource considerations**

***Patients will undergo enema FMT at the RAH and TQEH.***

***Microbiota analysis of biopsy and stool specimens will be performed at the CSIRO.***

***Data collection and analysis to be undertaken by Dr Lito Papanicolas. Enema FMT will be performed by Dr Sam Costello, Dr Robert Bryant, Dr Lito Papanicolas, Dr Renjy Nelson and Dr Morgyn Warner. Infection screen on blood and stool will be undertaken at the institute of medical and veterinary science laboratory. The costs for screening Stool Donors will be borne by Dr Sam Costello via an existing Grant. Dr Sam Costello, Dr Lito Papanicolas, Dr Robert Bryant, Dr Renjy Nelson and Dr Morgyn Warner’s time will be utilised for this study*.**

**Financial statement**

No conflicting interests are declared by the investigators.

**Investigators’ signatures**

***Dr LitoPapanicolas Dr Renjy Nelson Dr Morgyn Warner.***

*Dr Sam Costello* *Dr. Robert Bryant*



Research Ethics Committee

Tel: (08) 8222 4139
Fax: (08) 8222 3035
Email:
rah.ethics@health.sa.gov.au

**ROYAL ADELAIDE HOSPITAL**

North Terrace

Adelaide SA 5000

Tel: +61 8 8222 4000

Fax: +61 8 8222 5939

ABN 80 230 154 545

[www.rah.sa.gov.au](http://www.rah.sa.gov.au)