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Randomised, double-blind, placebo-controlled, phase 2, superiority trial to demonstrate the effectiveness of faecal microbiota transplantation for selective intestinal decolonisation of patients colonised by carbapenemase-producing *Klebsiella pneumoniae* (KAPEDIS)

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ABSTRACT

Introduction Infections caused by carbapenemase-producing *Enterobacterales* are frequent and associated with high rates of mortality. Intestinal carriers are at increased risk of infection by these microorganisms. Decolonisation strategies with antibiotics have not obtained conclusive results. Faecal microbiota transplantation (FMT) could be an effective and safe strategy to decolonise intestinal carriers of KPC-producing *Klebsiella pneumoniae* (KPC-Kp) but this hypothesis needs evaluation in appropriate clinical trials.

Methods and analysis The KAPEDIS trial is a single-centre, randomised, double-blind, placebo-controlled, phase 2, superiority clinical trial of FMT for eradication of intestinal colonisation by KPC-Kp. One hundred and twenty patients with rectal colonisation by KPC-Kp will be randomised 1:1 to receive encapsulated lyophilised FMT or placebo. The primary outcome is KPC-Kp eradication at 30 days. Secondary outcomes are: (1) frequency of adverse events; (2) changes in KPC-Kp relative load within the intestinal microbiota at 7, 30 and 90 days, estimated by real-time quantitative PCR analysis of rectal swab samples and (3) rates of persistent eradication, KPC-Kp infection and crude mortality at 90 days. Participants will be monitored for adverse effects throughout the intervention.

Ethics and dissemination Ethical approval was obtained from Reina Sofía University Hospital Institutional Review Board (approval reference number: 2019-003808-13). Trial results will be published in peer-reviewed journals and disseminated at national and international conferences.

Trial registration number NCT04760665.

Strengths and limitations of this study

- The double-blind, randomised, placebo-controlled design will control for spontaneous KPC-producing *Klebsiella pneumoniae* decolonisation.
- A remote, centralised, automatic randomisation system together with double-blinding will be implemented to reduce sources of potential bias.
- The trial is designed to evaluate the superiority of faecal microbiota transplantation against placebo in preventing multidrug-resistant infections.
- Concomitant administration of antibiotics during the follow-up period could act as confounder.
- The double-blind design is a strength of the study, while the single-centre design is a limitation.

INTRODUCTION

Multidrug-resistant bacteria represent an important threat to public health and particularly to vulnerable patient populations such as the elderly, the chronically ill, hospitalised patients, transplant and immunosuppressed recipients.[1–3] *Enterobacterales* are especially important from an antimicrobial resistance perspective, since they are a common cause of community-associated, as well as healthcare-associated infections. Carbapenem-resistant *Enterobacterales* (CRE) have been designated as a critical priority in the WHO Global Priority List for antimicrobial-resistant bacteria for the development of new antibiotics.

The gastrointestinal tract is a reservoir for antibiotic-resistant pathogens that cause disease by a variety of mechanisms. There is increasing evidence that the commensal microbiota have an indirect role in the control

of pathogen invasion by stimulating host immunity in the intestines.[4] Antibiotic treatment drastically alters the composition of the microbiota, interfering with this immunological balance, and promoting selection and proliferation of antibiotic-resistant pathogens.[4] Conversely, the commensal microbiota may be manipulated to prevent or cure infections caused by pathogenic bacteria, such as *Clostridium difficile* or multidrug-resistant organisms (MDRO), including vancomycin-resistant *Enterococcus faecium* and Gram-negative Enterobacterales.[4 5] So far, the most common control strategy for prevention of CRE infection in colonised patients is selective intestinal decolonisation (SDD) with oral, non-absorbable antibiotics, including colistin and aminoglycosides.[6–10] The reported decolonisation rates in observational studies range between 27.5% and 71%.[10 11] However, development of resistance to decolonising agents is frequently reported and there is a lack of randomised controlled trial (RCT) that allow adequate assessment of the effectiveness and safety of this strategy.[9] Considering these limitations, the clinical guidelines from the European Society of Clinical Microbiology and Infectious Diseases and European Committee on Infection Control do not recommend routine SDD of CRE carriers.[10]

Faecal microbiota transplantation (FMT) is an emerging therapy for targeting and modulating the human intestinal microbiota.[12] It has been demonstrated to be highly effective in patients with recurrent *Clostridioides difficile* infection (CDI) and has been incorporated into an European consensus document.[13] Promising results suggest that FMT may also be beneficial for the management of other disorders associated with gut microbiota dysbiosis. Recently, FMT has received attention as a potential decolonisation strategy for MDRO.[14–21] So far, a single RCT has evaluated whether oral antibiotics followed by FMT could eradicate intestinal carriage with extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-E, 72% of patients) or CRE (28% of patients).[16] The study failed to show non-inferiority of FMT, however, there were important limitations, including the lack of a placebo control, and failure to reach the targeted number of patients due to legislative impediments.[16] Besides this RCT, a recent meta-analysis evaluated five European studies (three case series and two case reports), and reported an overall 46% successful decolonisation rate at 1 month after FMT, with higher decolonisation rates for *P. aeruginosa* (100% decolonisation in four cases) as compared with New Delhi metallo-lactamase (NDM-1)-producing *Klebsiella pneumoniae* (Kp) (36.4%) and ESBL-producing Kp (40%).[22] In contrast, a recent prospective cohort study including 15 CRE carriers reported 60% eradication rates at 1 month after FMT.[20] In this study, Kp was the most common species (7/15) and *bla*KPC (Kp carbapenemase) was the most common carbapenemase gene (9/15), followed by *bla*OXA-48 (oxacillinase-48) (5/15) and *bla*NDM (1/15).[20] The observed differences in effectiveness of FMT for eradication of MDRO may be explained by differences in FMT conditions among studies, including bowel preparation before FMT, the donor, the dose and FMT preparation and administration procedures. Importantly, overall, studies report minor adverse events in patients who received FMT for MDRO eradication, and these include vomiting, diarrhoea, abdominal pain, and ileus.[22 23]

Despite all the limitations, the available evidence suggests a potential benefit of FMT as a decolonisation intervention for CRE, however, this needs to be confirmed by future well-designed RCTs. We have designed a phase II, double-blind, placebo-controlled clinical trial to assess the efficacy of oral FMT capsules to eradicate colonisation, with KPC carbapenemase-producing Kp (KPC-Kp).

METHODS AND ANALYSIS

Trial design and study setting

Randomised, double-blind, placebo-controlled, phase 2, superiority clinical trial with two parallel arms: 120 patients will be ramdomised 1:1 to receive FMT capsules (N=60) or placebo (N=60) (figure 1). Participants will be recruited from Reina Sofía University Hospital, a 1000-bed tertiary, academic, public hospital located in Cordoba, Spain. Some patients may be hospitalised at the time of recruitment and will thus be included during hospital stay. Participants who are not hospitalised or are discharged from hospital will be invited to attend the outpatient clinic. We followed Standard Protocol Items: Recommendations for Interventional Trials guidance, outlined in a 33-item checklist (online supplemental Annex 1) and figure 1.[24]

Primary objective

- To assess the efficacy of oral FMT capsules to eradicate intestinal colonisation by KPC-producing Kp at 30 days after FMT.

Primary outcome

- KPC-Kp eradication rate at 30 days in the intention-to-treat (ITT) population, including all randomised patients.

Secondary objectives

- To evaluate the safety of FMT.
- To determine if FMT is associated with an early (7 days post-FMT) and late (30 days post-FMT) decrease in the relative load of KPC-Kp within the intestinal microbiota.
- To evaluate if FMT is associated with persistent intestinal eradication at 3 months after intervention.
- To study if FMT is associated with a decrease in the incidence of KPC-Kp infections at 3 months after intervention.
- To evaluate if FMT is associated with a decrease in mortality due to KPC-Kp infections at 3 months after intervention.

	STUDY PERIOD						
	Enrolment	Allocation	Post-allocation				Close-out
TIMEPOINT	0 d	0 d	Visit 0 (0 d)	Visi1 (7-10 d)	Visit2 (30 ± 4 d)	Visit3 (90 ± 5 d)	90 d
ENROLMENT:							
Eligibility screen	X						
Informed consent	X						
Pregnancy test[1]	X						
Randomization	X						
Medical history / Anamnesis	X			X	X	X	
Physical examination[2]	X			X[3]	X[3]	X[3]	
Hemogram / Biochemistry[3]	X			X	X	X	
Serology[4]	X						
Rectal swab sample	X		X	X	X	X	
Recording of concomitant medication	X			X	X	X	
Dispensing control	X						
Allocation		X					
INTERVENTIONS:							
FMT			X				
Placebo			X				
ASSESSMENTS:							
Primary outcome							
KPC-Kp eradication					X		X
Secondary outcomes							
Adverse events			X	X	X	X	X
Changes in RL_{KPC}			X	X	X	X	X
Decolonization test			X	X	X	X	X
Persistent KPC-Kp eradication						X	X
Rate of KPC-Kp infections						X	X
Crude mortality						X	X

Figure 1 Schedule of enrolment, interventions and assessments according to SPIRIT guidelines. FMT, faecal microbiota transplantation; KPC-Kp, KPC-producing Klebsiella pneumoniae; SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials.

1 If female and of child-bearing age. 2 Physical examination: weight, height, blood pressure, heart and respiratory rate and temperature. Does not apply if interview is conducted telephonically.3 Hemogram with at least hemoglobin, white blood cell count, neutrophils and platelets. Blood chemistry at least with creatinine, urea, bilirubin, transaminases and PCR.4 Serology for hepatitis A, B and C viruses; human immunodeficiency virus (HIV), HIV-1 and HIV-2; nontreponemal rapid plasma reagin (RPR) test, and fluorescent treponemal antibody absorbed (FTA-ABS) test.

Secondary outcomes

- Proportion of patients with adverse events during follow-up: (1) reflux following FMT administration; (2) intolerable gastrointestinal side effects (ie, abdominal pain, flatulence, vomiting, constipation, diarrhoea or transient fever) leading to discontinuation of FMT before completing the study; (3) occurrence of any adverse/serious adverse effects.
- Changes in the relative load of KPC-Kp within the intestinal microbiota from day 0 (baseline) to days 7 (visit 1), 30 (visit 2) and 90 (visit 3), estimated by quantitative real-time PCR analysis (qPCR) of rectal swab samples (described below).
- Proportion of patients with persistent KPC-Kp eradication at 3 months of follow-up.
- Rate of KPC-Kp infections at 3 months.
- Crude mortality rate at 3 months.

Definitions

- Eradication: Negative rectal swab culture for KPC-Kp together with negative PCR *test for* bla_{KPC} gene. If the PCR result is positive, the subject is considered not-decolonised.
- Early decrease in intestinal KPC-Kp load: Significant reduction in the relative load of KPC-Kp within the gut microbiota in rectal swab samples obtained at day 7 of follow-up (visit 2) in patients receiving FMT versus placebo.
- Late decrease in intestinal KPC-Kp load: Significant reduction in the relative load of KPC-Kp within the gut microbiota in rectal swab samples obtained at day 30 of follow-up (visit 3) in patients receiving FMT vs placebo.
- Early decolonisation: Negative rectal swab culture for KPC-Kp and negative PCR *test for bla*KPC gene within 7–10 days of intervention.
- Persistent decolonisation: Negative rectal swab culture for KPC-Kp and negative PCR *test for bla*KPC gene on days 30 and 90 after the intervention.
- KPC-Kp infection: (1) Proven infection: KPC-Kp isolated from clinical specimens in the presence of clinical signs and symptoms of infection; (2) Probable infection: presence of clinical signs and symptoms of infection requiring treatment against KPC-Kp at the discretion of the attending physician, without isolation of KPC-Kp from clinical specimens.
- Crude mortality: All-cause mortality during follow-up.
- ITT population: all randomised patients.
- Per protocol population: Patients who meet the following criteria: (1) having been randomised; (2) complete data for the primary objective; (3) not having received antibiotics between randomisation and visit 3.
- Microbiologically evaluable population (PME): patients in whom all rectal colonisation studies have been performed during follow-up.

Patient eligibility criteria

Inclusion criteria

- Adult current or previous patients at Reina Sofía University Hospital with a positive rectal swab for KPC-Kp within 1 week before randomisation.
- The participant or legal representative must be able to provide written informed consent.
- Absence of KPC-Kp clinical samples at the time of informed consent and in the previous month.

Exclusion criteria

- Terminal illness or life expectancy of 3 months or less.
- Pregnancy or breast feeding.
- Inability/unwillingness to orally ingest study medication.
- Dysphagia and aspiration disorders.
- A history of colectomy, colostomy or ileostomy.
- Patients who have been treated with antibiotics within 30 days prior to consent.

- Absolute neutrophil count <500 /mm^3.
- Planned myelosuppressive chemotherapy within 30 days of randomisation, that is, dexamethasone, chemotherapy against solid tumours or prior to haematopoietic stem cell transplant (HSCT).
- HSCT within 30 days prior to consent.
- Clinical symptoms and signs of mucositis.
- Major abdominal surgery within the upcoming 30 days.
- Patients with Giannella Risk Score >12 puntos.[25]
- Selective digestive decolonisation with oral antibiotics within 3 months prior to randomisation.
- Severe food alergy.

Donor selection

General considerations

Donor selection and screening criteria for FMT is not currently standardised, showing variability among studies. In this RCT, we will use the exclusion criteria and conduct the microbiological studies suggested by García-García-de-Paredes *et al*[26] and Huttner *et al*.[16] To ensure double-blinding, only donors not related to the patients will be selected. This strategy has been shown to be safe and effective in studies where FMT was used as a treatment for *C. difficile* infection.[27 28] Initially, an interview and a questionnaire specifically designed for this purpose (online supplemental tables S1 and S2) will be carried out with the potential donor to identify the risk of diseases, especially those that may go unnoticed due to the unavailability of specific or sensitive diagnostic tests. Subsequently, a microbiological screening of the donor's blood and faeces as well as nasopharyngeal screening for Sars-CoV-2 will be performed on valid donors (online supplemental table S3). Based on expert recommendations, the pre-donation study will be carried out no longer than 4 weeks before donation.[13] This donor screening will be valid for 2 months after the first donation. After this period, microbiological screening will be repeated. If the same donor is required for a new donation period, the screening by questionnaire and all microbiological tests will be repeated.

Donor inclusion criteria

- To be aged between 18 and 60 years.
- To be in good health without significant past medical history.
- To have a normal body weight (body mass index between 20 and 25 kg/m^2).
- To have a stool with a normal appearance.
- To have an average stool frequency (1–3/day).
- Not to have an acute or chronic digestive disorder.

Donor exclusion criteria

- Infectious disease tests: HIV infection, hepatitis B and C, risk of transmission of HIV in the last 12 months, hepatitis B and C, risky sexual behaviours, use of illicit drugs, tattoos or piercings in the previous 6 months, current or prior history of stay in prison, current communicable disease, risk factors for Creutzfeldt-Jakob disease, travel in the last 6 months to countries with endemic diarrheal diseases or high risk of traveller's diarrhoea, history of *C. difficile* diarrhoea.
- Gastrointestinal comorbidities: inflammatory bowel disease, irritable bowel syndrome, chronic constipation or chronic diarrhoea, history of gastrointestinal malignancy or polyposis.
- Factors that can alter the intestinal microbiota: use of antibiotics in the last 3 months, use of immunosuppressants, glucocorticoids, calcineurin inhibitors, biological agents, use of antineoplastic drugs.
- Specific to the receptor: recent ingestion of an allergen to which the receptor is allergic. Others: previous major surgery of the digestive system, metabolic syndrome, diabetes mellitus, autoimmune diseases, connective tissue diseases, atopic diseases (asthma, eczema, eosinophilic pathologies of the gastrointestinal tract), chronic pain syndromes (fibromyalgia, chronic fatigue syndrome).

Microbiological studies

Rectal swab samples will be analysed for the presence of CRE, using both culture on selective chromogenic agar plates (CHROMID CARBA, bioMérieux, Marcy-l'Étoile, France) and qPCR.

For bacteria grown on culture, identification will be performed using MALDI- TOF mass spectrometry (Bruker, Germany) and carbapenemase production will be evaluated by a multiple strategy: (1) Antimicrobial susceptibility testing, with a first step using the commercial system MicroScan WalkAway and NC53 broth microdilution panels (Beckman Coulter, USA), and a second step, when a KPC-producing *K. pneumoniae* is identified, determining the Minimal Inhibitory Concentrations of ertapenem, imipenem, meropenem and other relevant agents (including ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam and meropenem-vaborbactam cefiderocol, fosfomycin, colistin, eravacycline) using EUMDROXF microdilution panels (Sensititre, Thermofisher, USA); clinical categories will be defined according to EUCAST breakpoints; (2) the Modified Carbapenem Inactivation Method, using meropenem discs[29]; (3) an immunochromatography test for the independent identification of OXA-48-like, KPC, NDM, imipenemase (IMP) and Verona integron-encoded metallo-beta-lactamase families of carbapenemases (NG-Test CARBA 5; NG Biotech, Guipry, France) and (4) conventional PCR for detection of the complete *blaKPC* gene, complemented with sequencing of the two DNA strands of corresponding amplicon when a positive result is obtained.

Quantification of the intestinal load of *blaKPC* gene in rectal swabs will be performed by qPCR. The load will be calculated relative to the total bacterial population (represented by the 16S rRNA gene) using the ΔΔCt method and pure cultures of KPC-producing *K. pneumoniae* as reference standards, as described in refs. 30 31.

Interventions

Trial interventions

Patients will be randomised 1:1 to receive oral capsules containing FMT or placebo. Mikrobiomik Healthcare Company S.L. (Vizcaya, Spain) will supply the FMT product (MBK-01), which consists of lyophilised microbiota encapsulated in hypromellose capsules (size 0), with a median mass of 250 g per capsule. Treatment will consist of 4 capsules, containing 1 g of lyophilised microbiota with ≥2 x 10^{11} total bacterial cells, obtained from a unique batch of lyophilised microbiota. Each batch of microbiota will be obtained from a minimum of 50 g donor faeces, based on previous studies supporting the efficacy of this dosing for treatment of CDI.[32] Participants in the placebo arm will receive four capsules containing microcystalline cellulose with the same shape, size and weight. The company will also supply the empty capsules to which the placebo will be added at the Pharmacy Service in our hospital. Capsules will be stored, with desiccant, at a temperature of 5°C±3°C, until they are dispensed. Mikrobiomik Healthcare Company will guarantee the traceability of the capsules and a record will be made of their storage, dispensing and destruction. Treatment will be dispensed to trial participants in presence of a member of the research team in a single dose in 1 day.

Concomitant care and interventions

Patients will fast for 12 hours and will receive a laxative preparation (one macrogol 3350, Movicol 13.8 g sachet dissolved in 125 mL water) the day before study intervention. The concomitant use of systemic antibiotics with activity against KPC-Kp at the time of intervention will not be allowed. Administration of these antibiotics during the study will be considered a proven or probable infection. During the follow-up period, administration of other decolonisation guidelines will not be allowed either. Other non-excluded drugs will be allowed.

Assignment of interventions

Allocation to treatment arms will be performed using a centralised, web-based automated randomisation system, integrated with the electronic case report file, and will be hosted by Maimonides Institute for Biomedical Research of Cordoba (Cordoba, Spain). After the patient's enrolment is confirmed, the randomisation specialist will assign a computer-generated random number to each patient. The randomisation data will be sent to a designated mailbox, and the responsible nurse will collect the treatment from the pharmacy at the hospital according to the assigned results. A double-blinded design will be used in this study for the physicians and statistical specialists, and patients and research assistants. However, the pharmacist will know the group of each patient. The allocation of the participants' treatment may be revealed at the end of the data analysis.

Evaluation during and after treatment

All patients will be followed for 90 days (±5 days) after the intervention or until death. Four follow-up visits will be scheduled for all participants at day 0 (baseline), day 7–10 (visit 1); day 30±4 (visit 2) and day 90±5 (visit 3) after end of intervention. The procedures that will be performed at each visit are indicated in figure 1**.** A rectal swab sample will be obtained at each visit for colonisation studies and quantification of KPC-Kp load by qPCR (see below). If a participant fails to be present at a scheduled visit, all attempts to contact them and any retrieved information will be recorded. A minimum of three documented contact attempts via phone calls will be performed, on separate occasions. All data collected will be included in an electronic database specifically designed for this study, with password-protected user authentication. To ensure the quality of the data, independent audits from investigators and sponsors may be carried out at any moment of the study.

Adverse effects

Adverse effects will be recorded and reported as part of routine follow-up. All events fulfilling the criteria of a serious adverse event that occur during the period of study will be reported to the promoter within 24 hours postevent occurrence. An insurance policy will be contracted to cover any harm from trial participation.

Sample size calculation

Sample size calculation was performed with G*Power V.3.1 program (https://gpower.software.informer.com/3.1/), assuming the following estimates: 90% power; 5% alpha error; decolonisation rate at 30 days of 30% in the control group based on a recent metanalysis reporting CRE colonisation rates of 76.7% (95% CI 64% to 81.8%) at 1 month in the absence of intervention[11]; decolonisation rate of 60% in the experimental group, based on a recently published study[18]; 1:1 treatment to placebo ratio; superiority considered if the CI lower bound for the difference between decolonisation rates in the experimental and control groups is greater than 5%; and expected informed consent rate of 40%. With these considerations, the sample size results in 112 patients. We added 7% more patients in order to account for possible loss to follow-up, resulting in a final sample size of 120 patients (60 patients in the experimental group and 60 patients in the control group). To reach the sample size, we will perform active surveillance of patients with KPC-Kp isolated from microbiological samples in our hospital.

Withdrawal from study

In accordance with the Declaration of Helsinki, patients have the right to withdraw from the study at any time and for any reason, communicating this decision personally or through their representative. The study withdrawal criteria will be the following: (1) at the request of the patient, through withdrawal of informed consent; (2) when the patient no longer complies with protocol

indications (protocol deviation); (3) as a result of any adverse event, regardless of its intensity, at the discretion of the investigator; (4) when for any reason the treatment is no longer safe for the patient; (5) as a result of an administrative decision taken by the researchers, sponsor or regulatory authority; (6) as a result of loss of contact during follow-up. If a patient is withdrawn from the trial prematurely, the investigator will register the main reason for the withdrawal in the clinical research file. Whenever necessary, the patient will continue to be followed, according to the standard protocols for treatment of their pathology, at the discretion of the responsible physician.

Statistical analysis

Frequencies and percentages of categorical variables, and median and interquartile ranges of continuous variables will be described. Comparisons will be performed using χ^2 or Fisher's exact test for categorial variables, and Student's t-test or Mann-Whitney U test for normally and not-normally distributed continuous variables, respectively.

The absolute difference in the percentages of decolonisation between the patients in the experimental and control groups, and its 95% CI, will be calculated. Clinically significant superiority will be considered if the 95% CI lower bound is greater than 5%. For the primary and secondary endpoints, the main analyses will be carried out in the ITT population. Then, an analysis will also be carried out in the per-protocol (PP) population (see definitions). All analyses will be performed using IBM SPSS V 20Statistics software.

ETHICS AND DISSEMINATION

The study is funded by Instituto de Salud Carlos III (Science and Innovation Ministry, Spanish government). It was authorised and approved by the ethical review board. Consent to participate will be obtained from all participants prior to the start of the trial by physicians included in our research team. The informed consent is provided as online supplemental Annex 2. All data will be anonymised. The study is being conducted in compliance with the protocol, regulatory requirements, International Council of Harmonisation E6 Good Clinical Practice and the ethical principles of the latest version of the Declaration of Helsinki, as adopted by the World Medical Association. Each substantial protocol amendment will be notified for approval to the relevant ethics committee(s) prior to implementation. All data collected will be kept strictly confidential and in accordance with all relevant legislation on control and protection of personal information. The participants will be identified on documentation by a unique ID number, not by name, in agreement with the European Regulation on data protection (EU 2016/679). All study-related information will be stored securely. The final results will be publicly disseminated regardless of the study outcomes. The results of this study will be published in peer-reviewed journals, as well as national and international conferences.

Patient and public involvement

Neither patients nor public authorities have been involved in the development of this study protocol.

DISCUSSION

In recent years, there has been a significant increase in the frequency of infections caused by carbapenem-producing *Enterobacterales* (CRE). These infections are associated with high mortality rates as a result of the difficulty in initiating effective empirical treatment and the limited therapeutic alternatives available for targeted treatment.[33 34] Rectal colonisation with CRE has previously been identified as an important risk factor for the development of subsequent CRE infection.[9 25 35 36] This situation has promoted efforts to prevent the acquisition and spread of these bacteria, including development of novel decolonisation strategies.

The utility of FMT for gut decolonisation of MDRO has been explored in several case reports, one prospective observational cohort and one RCT, summarised in a number of systematic reviews and metanalysis.[20 22 23 37 38] The only RCT, conducted by the R-GNOSIS study group, tested the efficacy of frozen capsulised FMT following a 5-day course of oral antibiotics in 39 carriers of CRE.[16] The desirability of pre-FMT antibiotic therapy in the context of MDRO decolonisation is unclear. Firstly, the administration of antibiotics renders it very difficult to unravel the independent contributions of antibiotics and FMT to CRE decolonisation. Secondly, preclinical studies with mouse models suggest that antibiotic preconditioning may improve the engraftment of specific taxa but not the overall engraftment of donor microbiota in the recipient mice.[39 40] Bar-Yoseph *et al*[20] reported that the use of antibiotics in the post-FMT period interfered with FMT engraftment among CRE-colonised recipients.[20]

Methods for FMT delivery include colonoscopy, nasoduodenal tub, colonic transendoscopic enteral tubing or oral capsules.[13 32 41] In this RCT, patients will be receiving FMT based on lyophilised oral capsules, which have been proven non-inferior to colonoscopy for the treatment of recurrent CDI and which also have higher acceptance by patients.[42] Further, patients with CRE colonisation who receive oral capsulised FMT achieved high eradication success (60%) at one month.[20] In addition, using lyophilised preparations facilitates capsule handling and stability, making it more feasible in hospital routine.

Regarding the amount of starting stool material, the European Consensus Conference on FMT in Clinical Practice for the treatment of *Clostridium difficile* infection (CDI) recommends a minimum of 30 g for the treatment of recurrent *CDI*[13]. Nevertheless, the optimal dose in FMT remains unclear since no randomised trials have compared different amounts of faecal matter so far. In the present RCT, the capsules with the lyophilised FMT

material will be provided by an external company, which has been legally authorised for production of the FMT capsules by the Spanish Agency for Medications and Healthcare Products. The company will guarantee that each treatment, consisting of a batch of four capsules, will contain a minimum of 2×10^{11} total bacterial cells obtained from a minimum of 30 g of feces.

The overall aim of this RCT is to evaluate the efficacy and safety of FMT for sustained eradication of CRE without using antibiotics that could impact the viability of the FMT content or confound results. It has been designed with placebo control to allow estimation of the contribution of spontaneous decolonisation to CRE eradication. If the efficacy and safety of FMT are proven, FMT may be considered a better approach for decolonisation of gut MDRO than selective antibiotics decolonisation, with lower ecological impact, and potentially reducing the risk of subsequent infections. A limitation of our study is that immunocompromised patients have been excluded. While there is increasing evidence of the beneficial effect of FMT for this patient population[43], given the single-centre nature of this RCT, they would be insufficiently represented to obtain statistically significant results that could justify their inclusion.

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REFERENCES

1 Aguado JM, Silva JT, Fernández-Ruiz M, *et al*. Management of multidrug resistant gram-negative bacilli infections in solid organ transplant recipients: SET/GESITRA-SEIMC/REIPI recommendations. *Transplant Rev* 2018;32:36–57.
2 Pérez-Nadales E, Gutiérrez-Gutiérrez B, Natera AM, *et al*. Predictors of mortality in solid organ transplant recipients with bloodstream infections due to carbapenemase-producing *Enterobacterales* : The impact of cytomegalovirus disease and lymphopenia. *Am J Transplant* 2020;20:1629–41.
3 van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 2017;8:460–9.
4 Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol* 2013;13:790–801.
5 Quraishi MN, Widlak M, Bhala N, *et al*. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Aliment Pharmacol Ther* 2017;46:479–93.
6 Saidel-Odes L, Polachek H, Peled N, *et al*. A randomized, double-blind, placebo-controlled trial of selective digestive decontamination using oral gentamicin and oral polymyxin E for eradication of carbapenem-resistant Klebsiella pneumoniae carriage. *Infect Control Hosp Epidemiol* 2012;33:14–19.
7 Oren I, Sprecher H, Finkelstein R, *et al*. Eradication of carbapenem-resistant Enterobacteriaceae gastrointestinal colonization with nonabsorbable oral antibiotic treatment: a prospective controlled trial. *Am J Infect Control* 2013;41:1167–72.
8 Lübbert C, Faucheux S, Becker-Rux D, *et al*. Rapid emergence of secondary resistance to gentamicin and colistin following selective digestive decontamination in patients with KPC-2-producing Klebsiella pneumoniae: a single-centre experience. *Int J Antimicrob Agents* 2013;42:565–70.
9 Machuca I, Gutiérrez-Gutiérrez B, Pérez Cortés S, *et al*. Oral decontamination with aminoglycosides is associated with lower risk of mortality and infections in high-risk patients colonized with colistin-resistant, KPC-producing Klebsiella pneumoniae. *J Antimicrob Chemother* 2016;71:3242–9.
10 Tacconelli E, Mazzaferri F, de Smet AM, *et al*. ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant gram-negative bacteria carriers. *Clin Microbiol Infect* 2019;25:807–17.
11 Bar-Yoseph H, Hussein K, Braun E, *et al*. Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. *J Antimicrob Chemother* 2016;71:2729–39.
12 Allegretti JR, Mullish BH, Kelly C, *et al*. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. *Lancet* 2019;394:420–31.

13 Cammarota G, Ianiro G, Tilg H, *et al*. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* 2017;66:569–80.
14 Seong H, Lee SK, Cheon JH, *et al*. Fecal microbiota transplantation for multidrug-resistant organism: efficacy and response prediction. *J Infect* 2020;81:719–25.
15 Saïdani N, Lagier J-C, Cassir N, *et al*. Faecal microbiota transplantation shortens the colonisation period and allows re-entry of patients carrying carbapenamase-producing bacteria into medical care facilities. *Int J Antimicrob Agents* 2019;53:355–61.
16 Huttner BD, de Lastours V, Wassenberg M, *et al*. A 5-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant Enterobacteriaceae: a randomized clinical trial. *Clin Microbiol Infect* 2019;25:830–8.
17 Dinh A, Fessi H, Duran C, *et al*. Clearance of carbapenem-resistant Enterobacteriaceae vs vancomycin-resistant enterococci carriage after faecal microbiota transplant: a prospective comparative study. *J Hosp Infect* 2018;99:481–6.
18 Bilinski J, Grzesiowski P, Sorensen N, *et al*. Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. *Clin Infect Dis* 2017;65:364–70.
19 Battipaglia G, Malard F, Rubio MT, *et al*. Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematologic malignancies carrying multidrug-resistance bacteria. *Haematologica* 2019;104:1682–8.
20 Bar-Yoseph H, Carasso S, Shklar S, *et al*. Oral Capsulized fecal microbiota transplantation for eradication of carbapenemase-producing Enterobacteriaceae colonization with a metagenomic perspective. *Clin Infect Dis* 2021;73:e166–75.
21 Singh R, de Groot PF, Geerlings SE, *et al*. Fecal microbiota transplantation against intestinal colonization by extended spectrum beta-lactamase producing Enterobacteriaceae: a proof of principle study. *BMC Res Notes* 2018;11:190.
22 Tavoukjian V. Faecal microbiota transplantation for the decolonization of antibiotic-resistant bacteria in the gut: a systematic review and meta-analysis. *J Hosp Infect* 2019;102:174–88.
23 Saha S, Tariq R, Tosh PK, *et al*. Faecal microbiota transplantation for eradicating carriage of multidrug-resistant organisms: a systematic review. *Clin Microbiol Infect* 2019;25:958–63.
24 Chan A-W, Tetzlaff JM, Altman DG, *et al*. Spirit 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200.
25 Cano A, Gutiérrez-Gutiérrez B, Machuca I, *et al*. Risks of infection and mortality among patients colonized with Klebsiella pneumoniae carbapenemase-producing K. pneumoniae: validation of scores and proposal for management. *Clin Infect Dis* 2018;66:1204–10.
26 García-García-de-Paredes A, Rodríguez-de-Santiago E, Aguilera-Castro L, *et al*. Trasplante de microbiota fecal. *Gastroenterología y Hepatología* 2015;38:123–34.
27 Youngster I, Russell GH, Pindar C, *et al*. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. *JAMA* 2014;312:1772–8.
28 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.
29 Pierce VM, Simner PJ, Lonsway DR, *et al*. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol* 2017;55:2321–33.
30 Lerner A, Adler A, Abu-Hanna J, *et al*. Spread of KPC-producing carbapenem-resistant *Enterobacteriaceae*: the importance of super-spreaders and rectal KPC concentration. *Clin Microbiol Infect* 2015;21:470.e1–470.e7.
31 Ramos-Ramos JC, Lázaro-Perona F, Arribas JR, *et al*. Proof-of-concept trial of the combination of lactitol with *Bifidobacterium bifidum* and *Lactobacillus acidophilus* for the eradication of intestinal OXA-48-producing *Enterobacteriaceae*. *Gut Pathog* 2020;12:15.
32 Reigadas E, Bouza E, Olmedo M, *et al*. Faecal microbiota transplantation for recurrent Clostridioides difficile infection: experience with lyophilized oral capsules. *J Hosp Infect* 2020;105:319–24.
33 Gutiérrez-Gutiérrez B, Salamanca E, de Cueto M, *et al*. Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing *Enterobacteriaceae* (INCREMENT): a retrospective cohort study. *Lancet Infect Dis* 2017;17:726–34.
34 Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, *et al*. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. *Clin Microbiol Rev* 2018;31:e00079–17.
35 Giannella M, Trecarichi EM, De Rosa FG, *et al*. Risk factors for carbapenem-resistant Klebsiella pneumoniae bloodstream infection among rectal carriers: a prospective observational multicentre study. *Clin Microbiol Infect* 2014;20:1357–62.
36 Tischendorf J, de Avila RA, Safdar N. Risk of infection following colonization with carbapenem-resistant Enterobactericeae: a systematic review. *Am J Infect Control* 2016;44:539–43.
37 Davido B, Batista R, Dinh A, *et al*. Fifty shades of graft: how to improve the efficacy of faecal microbiota transplantation for decolonization of antibiotic-resistant bacteria. *Int J Antimicrob Agents* 2019;53:553–6.
38 Woodworth MH, Hayden MK, Young VB. The role of fecal microbiota transplantation in reducing intestinal colonization with antibiotic-resistant organisms: the current landscape and future directions. *Open Forum Infect Dis* 2019;6:ofz288.
39 Freitag TL, Hartikainen A, Jouhten H, *et al*. Minor effect of antibiotic pre-treatment on the engraftment of donor microbiota in fecal transplantation in mice. *Front Microbiol* 2019;10:2685.
40 Ji SK, Yan H, Jiang T, *et al*. Preparing the gut with antibiotics enhances gut microbiota reprogramming efficiency by promoting Xenomicrobiota colonization. *Front Microbiol* 2017;8:1208.
41 Peng Z, Xiang J, He Z, *et al*. Colonic transendoscopic enteral tubing: a novel way of transplanting fecal microbiota. *Endosc Int Open* 2016;4:E610–3.
42 Kao D, Roach B, Silva M, *et al*. Effect of oral Capsule- vs Colonoscopy-Delivered fecal microbiota transplantation on recurrent Clostridium difficile infection: a randomized clinical trial. *JAMA* 2017;318:1985–93.
43 Alagna L, Palomba E, Mangioni D, *et al*. Multidrug-Resistant gram-negative bacteria decolonization in immunocompromised patients: a focus on fecal microbiota transplantation. *Int J Mol Sci* 2020;21:5619–22.