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Running title

Randomized clinical trial of fecal microbiota transplantation for decolonization of KPC-producing *Klebsiella pneumoniae*.

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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. Decolonization strategies with antibiotics have not obtained conclusive results. Fecal microbiota transplantation (FMT) could be an effective and safe strategy to decolonize 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-center, randomized, double-blind, placebo-controlled, phase 2, superiority clinical trial of FMT for eradication of intestinal colonization by KPC-Kp. One hundred and twenty patients with rectal colonization by KPC-Kp will be randomized 1:1 to receive encapsulated lyophilized FMT or placebo. The primary outcome is KPC-Kp eradication at 30 days. Secondary outcomes are: (i) frequency of adverse events; (ii) 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 (iii) 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 (2019-003808-13). Trial results will be published in peer-reviewed journals and disseminated at national and international conferences.

Trial registration number: Clinicaltrials.gov registration number NCT04760665.

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. The double-blind, randomized, placebo-controlled design will control for spontaneous KPC-Kp decolonization.
2. A remote, centralized, automatic randomization system together with double-blinding will be implemented to reduce sources of potential bias.
3. The trial is designed to evaluate the superiority of FMT against placebo in preventing multidrug-resistant infections.
4. Concomitant administration of antibiotics during the follow-up period could act as confounder.
5. The double-blind design is a strength of the study, while the single-center 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, hospitalized 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 World Health Organization (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. Conversely, the comensal microbiota may be manipulated to prevent or cure infections caused by pathogenic bacteria, particularly multidrug-resistant organisms (MDRO) such as vancomycin-resistant *Enterococcus faecium*, Gram-negative *Enterobacterales*, and *Clostridium difficile* [4,5]. So far, the most common control strategy for prevention of CRE infection in colonized patients is selective intestinal decolonization (SDD) with oral, non-absorbable antibiotics, including colistin and aminoglycosides [6–10]. The reported decolonization rates in observational studies range between 27.5% and 71% [10,11]. However, development of resistance to decolonizing agents is frequently reported and there is a lack of ramdomized clinical trials (RCT) that allow adequate assessment of the effectiveness and safety of this strategy. Considering these limitations, the clinical guidelines from the European Society of Clinical Microbiology and Infectious Diseases

and European Committee on Infection Control (ESCMID-ECIC) do not recommend routine SDD of CRE carriers [10].

Fecal microbiota transplantation (FMT) is an antibiotic-free decolonization strategy which has been demonstrated to be highly effective for treatment of recurrent *Clostridioides difficile* infections (CDI) [5]. It involves administration of fecal material containing distal gut microbiota from a healthy person (donor) to a patient with a disease or condition related to dysbiosis or alterations in the balance of their commensal microbiota. Recently, FMT has received attention as a potential decolonization strategy for MDRO [12–19]. So far, a single randomized control trial (RCT) has evaluated whether oral antibiotics followed by FMT could eradicate intestinal carriage with extended-spectrum beta-lactamase-producing *Enterobacterale*s (ESBL-E, 72% of patients) or carbapenemase-producing *Enterobacterales* (CPE, 28% of patients) [14]. 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 [14]. Besides this RCT, a recent meta-analysis evaluated five European studies (three case series and two case reports), and reported an overall 46% successful decolonization rate at one month after FMT, with higher decolonization rates for *P. aeruginosa* (100% decolonization in 4 cases) as compared to NDM-1-producing *Klebsiella pneumoniae* (36.4%) and ESBL-producing *Klebsiella pneumoniae* (40%) [20]. In contrast, a recent prospective cohort study including 15 CPE carriers reported 60% eradication rates at one month after FMT [18]. In this study, *Klebsiella pneumoniae* was the most common species (7/15) and *blaKPC* was the most common carbapenemase gene (9/15), followed by *blaOXA-48* (5/15) and *blaNDM* (1/15) [18]. 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, diarrhea, abdominal pain, and ileus [20,21].

Despite all the limitations, the available evidence suggests a potential benefit of FMT as a decolonization 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 colonization, with KPC carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp).

METHODS AND ANALYSIS

TRIAL DESIGN AND STUDY SETTING

Randomized, double-blind, placebo-controlled, phase 2, superiority clinical trial with two parallel arms: 120 patients will be ramdomized 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 hospitalized at the time of recruitment and will thus be included during hospital stay. Participants who are not hospitalized or are discharged from hospital will be invited to attend the outpatient clinic. We followed SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidance, outlined in a 33-item checklist (**Annex 1**) and **Figure 1** [22].

Primary objective

- To assess the efficacy of oral FMT capsules to eradicate intestinal colonization by KPC-producing *Klebsiella pneumoniae* at 30 days after FMT.

Primary outcome

- KPC-Kp eradication at 30 days in the intention-to-treat population, including all randomized 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.

Secondary outcomes

- Proportion of patients with adverse events during follow-up: (i) reflux following FMT administration; (ii) intolerable gastrointestinal side effects (i.e. abdominal pain, flatulence, vomiting, constipation, diarrhoea or transient fever) leading to discontinuation of FMT before completing the study; (iii) 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 polymerase chain reaction (PCR) test for *bla*$_{KPC}$ gene. If the PCR result is positive, the subject is considered not-decolonized.
- 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 versus placebo.
- Early decolonization: Negative rectal swab culture for KPC-Kp and negative polymerase chain reaction (PCR) test for *bla*$_{KPC}$ gene within 7-10 days of intervention.
- Persistent decolonization: Negative rectal swab culture for KPC-Kp and negative polymerase chain reaction (PCR) test for *bla*$_{KPC}$ gene on days 30 and 90 after the intervention.
- KPC-Kp infection: i) Proven infection: KPC-Kp isolated from clinical specimens in the presence of clinical signs and symptoms of infection; ii) 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.
- Intention-To-Treat (ITT) population: all randomized patients.

- Per protocol population (PPP): Patients who meet the following criteria: (i) having been randomized; (ii) complete data for the primary objective; (iii) not having received antibiotics between randomization and visit 3.
- Microbiologically evaluable population (PME): patients in whom all rectal colonization studies have been performed during follow-up.

Eligibility criteria

Inclusion criteria:

- Adult current or previous patients at Reina Sofía University Hospital with a positive rectal swab for KPC-Kp within one week before randomization.
- 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 breastfeeding.
- 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 randomization, i.e. dexamethasone, chemotherapy against solid tumors or prior to hematopoietic 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 [23].
- Selective Digestive Decolonization with oral antibiotics within 3 months prior to randomization.
- Severe food alergy.

Microbiological studies

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

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 MICs 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 [24]; (3) an immunochromatography test for the independent identification of OXA-48[-like], KPC, NDM, IMP and VIM 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 quantitative real-time PCR. 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 [25,26].

Interventions

Trial intervetions

Patients will be randomised 1:1 to receive oral capsules containing TMF 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 a batch of 4 capsules, containing 1 g of lyophilized microbiota with ≥ 2 x 10^{11} total bacterial cells, obtained from a minimum of 30 g donor feces. Participants in the placebo arm will receive 4 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 ± 3ºC, 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 one 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 decolonization 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 centralized, web-based automated randomization system, integrated with the electronic case report file (eCRF), and will be hosted by Maimonides Institute for Biomedical Research of Cordoba (Cordoba, Spain). After the patient's enrolment is confirmed, the randomization specialist will assign a computer-generated random number to each patient. The randomization 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 colonization 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 post event 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 3.1 program (https://gpower.software.informer.com/3.1/), assuming the following estimates: 90% power; 5% alpha error; decolonization rate at 30 days of 30% in the control group and 60% in the experimental group; 1:1 treatment to placebo ratio; superiority considered if the confidence interval lower bound for the difference between decolonization rates in the experimental and control groups is greater than 5%; and expected informed consent rate of 40%. The estimated decolonization rate in patients treated with FMT has been obtained from a recently published study [16] . The proportion of decolonized patients in the control group was obtained from a previous prospective observational cohort in our center of patients with intestinal colonization by KPC-Kp (KLEBCOM study, unpublished results). 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: a) at the request of the patient, through withdrawal of informed consent; b) when the patient no longer complies with protocol indications (protocol deviation); c) as a result of any adverse event, regardless of its intensity, at the discretion of the investigator; d) when for any reason the treatment is no longer safe for the patient; e) as a result of an administrative decision taken by the researchers, sponsor, or regulatory authority; f) 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 Chi-square or Fisher's test for categorial variables, and Student's T or Mann-Whitney U test for normally and not-normally distributed continuous variables, respectively.

The absolute difference in the percentages of decolonization between the patients in the experimental and control groups, and its 95% confidence interval, will be calculated. Clinically significant superiority will be considered if the 95% confidence interval lower bound is

greater than 5%. For the primary and secondary endpoints, the main analyses will be carried out in the intention-to-treat (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 Statistics software.

ETHICS AND DISSEMINATION

The study is funded by Instituto de Salud Carlos III (Science and Innovation Ministry, Spanish government). It was authorized 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 **Annex 2**. All data will be anonymized. The study is being conducted in compliance with the protocol, regulatory requirements, International Council of Harmonization (ICH) 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. The trial is registered with ClinicalTrials.gov as NCT04760665 (February 18, 2021). 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 [27,28]. Rectal colonization with CRE has previously been identified as an important risk factor for the development of subsequent CRE infection [9,23,29,30]. This situation has promoted efforts to prevent the acquisition and spread of these bacteria, including development of novel decolonization strategies.

The utility of fecal microbiota transplantation (FMT) for gut decolonization of multidrug resistant organisms (MDROs) has been explored in several case reports, one prospective observational cohort and one RCT, summarized in a number of systematic reviews and metanalysis [18,20,21,31,32]. The only RCT, conducted by the R-GNOSIS study group, tested the efficacy of frozen capsulized FMT following a 5-day course of oral antibiotics in 39 carriers of extended spectrum β-lactamase *Enterobacterales* (ESBL-E) and carbapenemase-producing *Enterobacteales* (CPE) [14]. The desirability of pre-FMT antibiotic therapy in the context of MDRO decolonization is unclear. Firstly, the administration of antibiotics renders it very difficult to unravel the independent contributions of antibiotics and FMT to CRE decolonization. Secondly, pre-clinical studies with mouse models suggest that antibiotic pre-conditioning may improve the engraftment of specific taxa but not the overall engraftment of donor microbiota in the recipient mice [33,34]. Bar-Yoseph *et al* [18] reported that the use of antibiotics in the post-FMT period interfered with FMT engraftment among CPE-colonized recipients [18].

In this RCT, patients will be receiving FMT based on lyophilized oral capsules, which have been proven non-inferior to colonoscopy for the treatment of recurrent CDI and which also have higher acceptance by patients (Kao et al. 2017). Further, patients with CPE colonization who receive oral capsulized FMT achieved high eradication success (60%) at one month [18]. In addition, using lyophilized 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 Faecal Microbiota Transplantation in Clinical Practice for the treatment of *Clostridium difficile* infection (CDI) recommends a minimum of 30 g for the treatment of recurrent *CDI* [35]. Nevertheless, the optimal dose in FMT remains unclear since no randomized trials have compared different amounts of faecal matter so far. In the present RCT, the capsules with the lyophilized FMT material will be provided by an external company, which has been legally authorized for production of the FMT capsules by the Spanish Agency for Medications and Healthcare Products (AEMPS). The company will guarantee that each treatment, consisting of a batch of 4 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 CPE 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 decolonization to CPE eradication. If the efficacy and safety of FMT are proven, FMT may be considered a better approach for decolonization of gut MDRO than selective antibiotics decolonization, with lower ecological impact, and potentially reducing the risk of subsequent infections.

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CONTRIBUTIONS

JCO conceived and designed the study. JCO obtained funding for the research. JCO is the Study Coordinator. EPN and LMM will coordinate microbiological studies. EPN and JCO drafted the manuscript. All other authors will be directly involved in different aspects of this RCT and have reviewed, edited and approved the final version of the paper.

TRIAL STATUS

Current protocol approved is version 1.0 dated 4th September 2019. Recruitment will begin on 5th October 2021 and will end on 5th October 2023.

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COMPETING INTERESTS STATEMENT

Juan José Castón reports personal fees from Merck for educational purposes and a research grant from Pfizer outside the submitted work. All other authors declare that they have no competing interests.

PATIENTS CONSENT FOR PUBLICATION

Not required

Figure 1. Schedule of enrolment, interventions, and assessments according to SPIRIT guidelines.

	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

Abbreviations: d, days; FMT, Fecal Microbiota Transplantation; $RL_{KPC,}$ relative intestinal load of bla_{KPC}.

[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.

Annex 1. SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents.

Section/item	Item No	Description	Page in the document
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	--
Protocol version	3	Date and version identifier	24
Funding	4	Sources and types of financial, material, and other support	24
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 24
	5b	Name and contact information for the trial sponsor	--
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	--
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7,8
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	10-11
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12-13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	14-15
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	12, Figure 1
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-13

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7,8, Figure 1
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14-15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	14-15
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Figure 1
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14-15
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14-15
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15-16
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	15-16
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomised analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	15-16
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	--
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	--

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	16
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Non-applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	14
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
	31b	Authorship eligibility guidelines and any intended use of professional writers	--
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	--
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Annex 2
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Non-applicable

HOJA DE INFORMACIÓN AL PACIENTE

Título del Estudio	Ensayo clínico aleatorizado, de superioridad, doble ciego, controlado con placebo, en fase II, para demostrar la eficacia del trasplante de microbiota fecal en la descolonización intestinal selectiva de los pacientes colonizados por *Klebsiella pneumoniae* productora de carbapenemasa tipo KPC
Código del estudio	KAPEDIS
Promotor	Fundación para la Investigación Biomédica de Córdoba
Investigador principal	Juan José Castón Osorio
Centro	Hospital Universitario Reina Sofía de Córdoba

Introducción

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que se le invita a participar. El estudio ha sido aprobado por un Comité de Ética de la Investigación con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo a la legislación vigente, el Real Decreto 1090/2015 de 4 de diciembre y el Reglamento Europeo 536/2014 de 16 de abril, por los que se regulan los ensayos clínicos con medicamentos.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda decidir si acepta o no participar en este estudio. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir.

Además, puede consultar con las personas que considere oportuno.

Participación voluntaria

Le invitamos a participar en el estudio porque usted presenta una colonización por una bacteria multirresistente (*Klebsiella pneumoniae* productora de carbapenemasa KPC). Debe saber que su participación en este estudio es voluntaria y que puede decidir NO participar. Si decide participar, puede cambiar su decisión y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su atención sanitaria.

Objetivo del estudio

El objetivo principal del estudio es determinar si el trasplante de microbiota intestinal es eficaz para la descolonización intestinal en pacientes colonizados por *Klebsiella pneumoniae* portadora de KPC.

Descripción del estudio

Este estudio pretende incluir un total de 120 pacientes con colonización intestinal por *Klebsiella pneumoniae* portadora de KPC. Se entiende por colonización intestinal la presencia de esta bacteria en la flora intestinal.

En todos los seres humanos habitan de forma natural millones de microorganismos, sobre todo bacterias, y fundamentalmente en el intestino. Para mantener el estado de salud de un individuo es necesario un complejo equilibrio entre estas bacterias. La edad avanzada, la depresión del sistema inmune y la toma de antibióticos son factores que alteran el ecosistema microbiológico del intestino. Se ha comprobado que la ruptura de este delicado equilibrio favorece la proliferación de bacterias causantes de enfermedad como *Clostridium difficile*.

El trasplante de microbiota intestinal consiste en la introducción de una muestra de bacterias intestinales procedentes de un donante sano en el tracto gastrointestinal de otra persona. Ello podría permitir la sustitución de las bacterias resistentes del intestino del receptor (usted) por las bacterias del intestino del donante. Para realizar el trasplante, en primer lugar se somete al receptor, al donante y a la muestra de material fecal a una serie de pruebas diagnósticas para garantizar la seguridad biológica del proceso de las partes implicadas. Seguidamente las heces se procesan en el laboratorio hasta obtener un líquido claro libre de material de desecho, que se llama sobrenadante. Por último, este sobrenadante se administra en el intestino grueso del enfermo mediante cápsulas que son ingeridas por el receptor. El fundamento para ello es que la flora intestinal normal procedente del donante sustituya a las bacterias resistentes que están presentes en su intestino.

El trasplante de microbiota intestinal está actualmente indicado en la infección por *Clostridium difficile* recurrente. Además de esta indicación, en la actualidad el trasplante de microbiota está siendo estudiado para comprobar si es eficaz para la descolonización de pacientes con colonización intestinal por bacterias resistentes a antibióticos.

Si acepta participar en nuestro estudio usted será asignado de forma aleatoria (al azar) a recibir cápsulas que contienen microbiota intestinal o placebo (una cápsula con el mismo

aspecto que la que contiene microbiota intestinal, pero que no es farmacológicamente activa y por tanto no se espera que tenga efecto). Usted tendrá un 50% de probabilidades de entrar en cada grupo del estudio. Ni el médico ni usted sabrán cúal es el tratamiento que va a recibir.

Actividades del estudio

Su periodo de participación en el estudio será de 90 días. Esta participación se divide en dos fases, por un lado la fase de tratamiento que será el primer día tras su inclusión en el estudio y posteriormente una fase de seguimiento hasta el día 90 después del tratamiento. En la fase de tratamiento usted recibirá 4-5 cápsulas que contendrán trasplante de microbiota o placebo (una sustancia inocua y sin efecto terapéutico). El día previo a la toma de las cápsulas usted recibirá una prepación laxante (un sobre de macrogol disuelto en 125 ml de agua).

En total se realizarán 4 visitas, con la periodicidad establecida en la tabla adjunta. Se realizará una primera visita de selección (día 0), entre 7 y 10 días después la visita 1, la visita de prueba de descolonización que se realizará alrededor (entre 4 días antes o después) de los 30 días después de la visita de selección, y la visita 3 que se efectuará alrededor (entre 5 días antes o después) de los 90 días tras la visita de selección.

Estas visitas no se llevarían a cabo en caso de no participar en el estudio, aunque pueden realizarse en su domicilio sin que tenga que desplazarse al hospital si es posible y usted lo desea.

Durante el estudio se le tomarán muestras de sangre y de frotis rectal las cuales se realizarán de forma extraordinaria por su participación en el estudio.

Tabla 1. Calendario de visitas

Procedimientos	Visita selección (Día 0)	Visita 1 (Día 7-10)	Visita 2. Prueba de descolonización (Día 30 ±4)	Visita 3 (Día 90 ±5)
Consentimiento informado	X			
Criterios inclusión / exclusión	X			
Test de embarazo	X			
Aleatorización	X			
Historia clínica / Anamnesis	X	X	X	X
Exploración física	X	X	X	X
Analítica de sangre	X	X	X	X
Serología	X			
Frotis rectal	X	X	X	X
Medicación concomitante	X	X	X	X
Control de dispensación	X			
Acontecimientos adversos	X	X	X	X

Riesgos y molestias derivados de su participación en el estudio

El trasplante de microbiota intestinal es un procedimiento autorizado en nuestro país para el tratamiento de la diarrea recurrente por la bacteria *Clostridium difficile*. En la actualidad su uso para la descolonización intestinal por bacterias resistentes no está aprobada aún en nuestro país encontrándose en fase experimental.

En los estudios realizados hasta la fecha el trasplante de microbiota se ha mostrado como una estrategia segura, no obstante, estos no pueden excluirse por completo efectos secundarios a largo plazo.

El donante puede presentar alguna enfermedad o infección no encontrada en el momento de su estudio a la que el receptor puede estar expuesto tras el trasplante de microbiota intestinal. Todos los donantes siguen un proceso de selección exhaustivo previo a la toma de la muestra para prevenirle de ser expuesto a alguna enfermedad o infección. Aunque sería extremadamente raro, no podemos descartar por completo ese riesgo.

Algunos pacientes experimentan un breve cuadro de diarrea tras el procedimiento, que suele ceder en las primeras 24-48 horas. Otros síntomas que pueden presentarse con menor frecuencia el día de la intervención son sensación de décimas de fiebre, hinchazón, flatulencia y dolor o molestias abdominales difusas.

Para conocer más sobre los posibles efectos no deseados de este procedimiento consulte al médico del estudio.

Si usted acepta participar en el estudio se le realizarán pruebas (análisis de sangre y heces) antes de proceder al trasplante y posteriormente según el calendario de visitas expuesto en la tabla 1.

Como riesgo derivado de la extracción de sangre puede producirse un pequeño hematoma, puede haber dolor local, hemorragia y muy excepcionalmente, puede producirse infección en el punto donde se extrae la sangre.

Como participante en el estudio tiene la responsabilidad de cumplir todas las visitas y actividades del estudio. También deberá notificar cualquier evento que le suceda o cambios en la medicación en caso de urgencia, ya que no podrá modificarla por su cuenta ni tomarla junto a “plantas medicinales” sin consultar antes con el médico del estudio.

Posibles beneficios

Si demostramos la hipótesis de este estudio, usted habrá contribuido a mejorar las estrategias de descolonización en otros pacientes que como usted, están colonizados por la bacteria *Klebsiella pneumoniae* portadora de KPC. Nuestra estrategia puede permitir evitar el empleo de antibióticos para la descolonización, evitando la aparición de efectos secundarios y de aparición de bacterias resistentes a los antibióticos administrados. No obstante, es posible que no obtenga ningún beneficio para su salud por participar en este estudio.

Advertencia relativa al embarazo

En nuestro estudio no se incluye la participación de mujeres embarazadas. Las mujeres en edad fértil requerirán la realización de un test de gestación, el cual deberá ser negativo para su inclusión en el estudio.

Si usted es mujer, en caso de quedarse embarazada durante su participación en el estudio debe informar a su médico de inmediato para recibir la asistencia médica adecuada. En la actualidad desconocemos los efectos que puede conllevar el trasplante de microbiota fecal sobre el feto.

En caso de producirse un embarazo, se le solicitará la recogida de datos del mismo y de datos de salud de su bebé durante los 12 meses posteriores al nacimiento. Toda la información relativa a su embarazo será tratada de acuerdo a la normativa de protección de datos vigente.

Tratamientos alternativos

Actualmente no hay una estrategia definida de forma general para la descolonización intestinal de los pacientes colonizados por bacterias resistentes. Para intentar esta descolonización se emplean distintos antibióticos de forma oral como gentamicina o colistina cuya dosis y tiempo de administración varían en cada centro. Su médico del estudio le dará más información si lo desea.

Seguro

El Promotor del estudio dispone de una póliza de seguros que se ajusta a la legislación vigente (Real Decreto 1090/2015) y que le proporcionará la compensación e indemnización en caso de menoscabo de su salud o de lesiones que pudieran producirse en relación con su participación en el estudio, siempre que no sean consecuencia de la propia enfermedad que se estudia o de la evolución propia de su enfermedad como consecuencia de la ineficacia del tratamiento.

Si desea más información relativa a este apartado, consulte con el investigador principal del estudio de su centro.

Le informamos que es posible que su participación en este ensayo clínico pueda modificar las condiciones generales y particulares (cobertura) de sus pólizas de seguros (vida, salud, accidente…). Por ello le recomendamos que se ponga en contacto con su aseguradora para determinar si la participación en este estudio afectará a su actual póliza de seguros.

Protección de datos personales

El promotor se compromete al cumplimiento de la Ley Orgánica 3/2018, de 5 de diciembre, de protección de datos personales y garantía de los derechos digitales, así como el Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 de abril de 2016 relativo a la protección de las personas físicas en lo que respecta al tratamiento de datos personales y a la libre circulación de estos datos (Reglamento general de protección de datos).

Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no se incluya información que pueda identificarle y sólo su médico del estudio/colaboradores

podrá relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna salvo excepciones en caso de urgencia médica o requerimiento legal. El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los participantes se ajustarán a lo dispuesto en esta ley.

El acceso a su información personal identificada quedará restringido al médico del estudio/colaboradores, a autoridades sanitarias (Agencia Española de Medicamentos y Productos Sanitarios, autoridades sanitarias extranjeras), al Comité de Ética de la Investigación y al personal autorizado por el promotor (monitores del estudio, auditores), cuando lo precisen para comprobar los datos personales, los procedimientos del estudio clínico y el cumplimiento de las normas de buena práctica clínica (siempre manteniendo la confidencialidad de la información de acuerdo a la legislación vigente).

Los datos se recogerán en un fichero de investigación responsabilidad de la institución y se tratarán en el marco de su participación en este estudio. El promotor adoptará las medidas pertinentes para garantizar la protección de su privacidad y no permitirá que sus datos se crucen con otras bases de datos que pudieran permitir su identificación.

De acuerdo a lo que establece la legislación de protección de datos, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos. También puede limitar el tratamiento de datos que sean incorrectos, solicitar una copia o que se trasladen a un tercero (portabilidad) los datos que usted ha facilitado para el estudio.

Para ejercitar sus derechos, diríjase al investigador principal del estudio o al Delegado de Protección de Datos del promotor en dpd@imibic.org. Le recordamos que los datos no se pueden eliminar aunque deje de participar en el ensayo para garantizar la validez de la investigación y cumplir con los deberes legales y los requisitos de autorización de medicamentos. Así mismo tiene derecho a dirigirse a la Agencia de Protección de Datos si no queda satisfecho.

Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos, pero sí se utilizarán los que ya se hayan recogido.

El Investigador y el Promotor están obligados a conservar los datos recogidos para el estudio al menos hasta 25 años tras su finalización. Posteriormente, su información personal sólo se conservará por el centro para el cuidado de su salud y por el promotor para otros fines de

investigación científica si usted hubiera otorgado su consentimiento y si así lo permite la ley y requisitos éticos aplicables.

Si realizáramos transferencia de sus datos codificados fuera de la UE a las entidades de nuestro grupo, a prestadores de servicios o a investigadores científicos que colaboren con nosotros, los datos del participante quedarán protegidos con salvaguardas tales como contratos u otros mecanismos establecidos por las autoridades de protección de datos. Si quiere saber más al respecto, puede contactar al Delegado de Protección de Datos del promotor en dpd@imibic.org.

Gastos y compensación económica

El promotor del estudio es el responsable de gestionar la financiación del mismo. Para la realización del estudio el promotor del mismo ha firmado un contrato con el médico del estudio y centro donde se va a realizar.

Ni el investigador ni el centro reciben ninguna compensación económica derivada del estudio.

Usted no tendrá que pagar por los medicamentos ni por pruebas específicas del estudio. Su participación en el estudio no le supondrá ningún gasto adicional a la práctica clínica habitual.

Otra información relevante

Una descripción de este ensayo clínico estará disponible en https://reec.aemps.es, según exige la legislación española, así como en https://clinicaltrials.gov.

Cualquier nueva información referente al producto en investigación utilizado en el estudio y que pueda afectar a su disposición para participar en el estudio, que se descubra durante su participación, le será comunicada por su médico lo antes posible.

Debe saber que puede ser excluido del estudio si el promotor o los investigadores del estudio lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca por la medicación en estudio o porque consideren que no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo que ha ocasionado su retirada del estudio.

Al firmar la hoja de consentimiento adjunta, se compromete a cumplir con los procedimientos del estudio que se le han expuesto.

Si como participante en el estudio dejara de acudir a las visitas sin retirar el consentimiento, el promotor podrá realizar un seguimiento de usted.

Debe usted saber que es posible que su médico de Atención Primaria tenga conocimiento de su participación en este estudio.

¿Qué tratamiento recibiré cuando finalice el ensayo clínico?

Cuando acabe su participación recibirá el mejor tratamiento disponible y que su médico considere el más adecuado para su enfermedad, pero es posible que no se le pueda seguir administrando el producto en investigación del estudio. Por lo tanto, ni el investigador ni el promotor adquieren compromiso alguno de mantener dicho tratamiento fuera de este estudio.

Contacto en caso de dudas

Si durante su participación tiene alguna duda o necesita obtener más información, póngase en contacto con el Dr._____
del servicio de_____en el teléfono _____.

CONSENTIMIENTO INFORMADO DEL PARTICIPANTE

Título del estudio: Ensayo clínico aleatorizado, de superioridad, doble ciego, controlado con placebo, en fase II, para demostrar la eficacia del trasplante de microbiota fecal en la descolonización intestinal selectiva de los pacientes colonizados por *Klebsiella pneumoniae* productora de carbapenemasa tipo KPC

Código de protocolo: KAPEDIS. **Versión del protocolo:** 1.0 de 4-septiembre-2019

Yo (nombre y apellidos del participante):_____

- He leído la hoja de información que se me ha entregado sobre el estudio.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- He hablado con (nombre del investigador):_____
- Comprendo que mi participación es voluntaria.
- Comprendo que puedo retirarme del estudio:
  - Cuando quiera.
  - Sin tener que dar explicaciones.
  - Sin que esto repercuta en mis cuidados médicos.

Deseo que me comuniquen la información derivada de la investigación que pueda ser relevante para mi salud: SÍ ☐ NO ☐

Recibiré una copia firmada y fechada de este documento de consentimiento informado. Presto libremente mi conformidad para participar en el estudio.

Nombre del participante: Nombre del investigador:

Fecha:_____/_____/_____ Fecha:_____/_____/_____

Firma del participante: Firma del investigador:

Cuando se obtenga el CI en personas con capacidad modificada para dar su CI. Nombre del representante leal o familiar: Nombre del investigador:

Fecha:_____/_____/_____ Fecha:

_____/_____/_____

Firma del representante leal: Firma del investigador:

CONSENTIMIENTO INFORMADO DEL PACIENTE ANTE TESTIGOS

Título del estudio: Ensayo clínico aleatorizado, de superioridad, doble ciego, controlado con placebo, en fase II, para demostrar la eficacia del trasplante de microbiota fecal en la descolonización intestinal selectiva de los pacientes colonizados por *Klebsiella pneumoniae* productora de carbapenemasa tipo KPC

Código de protocolo: KAPEDIS. **Versión del protocolo:** 1.0 de 4-septiembre-2019

Yo (nombre y apellidos del testigo):_____
como testigo, afirmo que en mi presencia se ha informado a D/Dª (nombre y apellidos del participante)_____
y se ha leído o le han leído la hoja de información que se le ha entregado sobre el estudio, de modo que:

- Ha podido hacer preguntas sobre el estudio.
- Ha recibido suficiente información sobre el estudio.
- Ha hablado con (nombre del investigador):_____
- Comprende que su participación es voluntaria.
- Comprende que puede retirarse del estudio:
  - Cuando quiera.
  - Sin tener que dar explicaciones.
  - Sin que esto repercuta en sus cuidados médicos.

Recibirá una copia firmada y fechada de este documento de consentimiento informado.
El participante desea que se le comunique la información derivada de la investigación que pueda ser relevante para su salud: SÍ ☐ NO ☐

Nombre del testigo: Nombre del investigador:

Fecha:_____/_____/_____ Fecha:

_____/_____/_____

Firma del testigo: Firma del investigador:

El participante del estudio ha indicado que no puede leer / escribir. Un miembro del personal del estudio le ha leído el documento de consentimiento, lo ha revisado y comentado con el participante y se le ha concedido la oportunidad de hacer preguntas o consultarlo con otras personas. El testigo es una persona imparcial, ajena al estudio.

BMJ Open

A randomized, double-blind, placebo-controlled, phase 2, superiority trial to demonstrate the effectiveness of fecal microbiota transplantation for Selective Intestinal Decolonization of patients colonized by carbapenemase-producing *Klebsiella pneumoniae* (KAPEDIS).

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A randomized, double-blind, placebo-controlled, phase 2, superiority trial to demonstrate the effectiveness of fecal microbiota transplantation for **S**elective **I**ntestinal **D**ecolonization of patients colonized by carbapenemase-producing **K**lebsiella **P**neumoniae (KAPEDIS)

Running title

Randomized clinical trial of fecal microbiota transplantation for decolonization of KPC-producing *Klebsiella pneumoniae*.

Authors

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Keywords

fecal microbiota transplantation, selective intestinal decolonization, *Klebsiella pneumoniae*, carbapenemase.

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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. Decolonization strategies with antibiotics have not obtained conclusive results. Fecal microbiota transplantation (FMT) could be an effective and safe strategy to decolonize 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-center, randomized, double-blind, placebo-controlled, phase 2, superiority clinical trial of FMT for eradication of intestinal colonization by KPC-Kp. One hundred and twenty patients with rectal colonization by KPC-Kp will be randomized 1:1 to receive encapsulated lyophilized FMT or placebo. The primary outcome is KPC-Kp eradication at 30 days. Secondary outcomes are: (i) frequency of adverse events; (ii) 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 (iii) 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: Clinicaltrials.gov registration number NCT04760665.

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. The double-blind, randomized, placebo-controlled design will control for spontaneous KPC-Kp decolonization.
2. A remote, centralized, automatic randomization system together with double-blinding will be implemented to reduce sources of potential bias.
3. The trial is designed to evaluate the superiority of FMT against placebo in preventing multidrug-resistant infections.
4. Concomitant administration of antibiotics during the follow-up period could act as confounder.
5. The double-blind design is a strength of the study, while the single-center 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, hospitalized 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 World Health Organization (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 colonized patients is selective intestinal decolonization (SDD) with oral, non-absorbable antibiotics, including colistin and aminoglycosides [6–10]. The reported decolonization rates in observational studies range between 27.5% and 71% [10,11]. However, development of resistance to decolonizing agents is frequently reported and there is a lack of ramdomized clinical trials (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 (ESCMID-ECIC) do not recommend routine SDD of CRE carriers [10].

Fecal microbiota transplantation 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 decolonization strategy for MDRO [14–21]. So far, a single randomized control trial (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 carbapenem-resistant *Enterobacterales* (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 decolonization rate at one month after FMT, with higher decolonization rates for *P. aeruginosa* (100% decolonization in 4 cases) as compared to New Delhi metallo-lactamase (NDM-1)-producing *Klebsiella pneumoniae* (36.4%) and ESBL-producing *Klebsiella pneumoniae* (40%) [22]. In contrast, a recent prospective cohort study including 15 CRE carriers reported 60% eradication rates at one month after FMT [20]. In this study, *Klebsiella pneumoniae* was the most common species (7/15) and *bla*KPC *(Klebsiella pneumoniae* 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, diarrhea, abdominal pain, and ileus [22,23].

Despite all the limitations, the available evidence suggests a potential benefit of FMT as a decolonization 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 colonization, with KPC carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp).

METHODS AND ANALYSIS

TRIAL DESIGN AND STUDY SETTING

Randomized, double-blind, placebo-controlled, phase 2, superiority clinical trial with two parallel arms: 120 patients will be ramdomized 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 hospitalized at the time of recruitment and will thus be included during hospital stay. Participants who are not hospitalized or are discharged from hospital will be invited to attend the outpatient clinic. We followed SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidance, outlined in a 33-item checklist (**Annex 1**) and **Figure 1** [24].

Primary objective

- To assess the efficacy of oral FMT capsules to eradicate intestinal colonization by KPC-producing *Klebsiella pneumoniae* at 30 days after FMT.

Primary outcome

- KPC-Kp eradication rate at 30 days in the intention-to-treat population, including all randomized 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.

Secondary outcomes

- Proportion of patients with adverse events during follow-up: (i) reflux following FMT administration; (ii) intolerable gastrointestinal side effects (i.e., abdominal pain, flatulence, vomiting, constipation, diarrhoea, or transient fever) leading to discontinuation of FMT before completing the study; (iii) 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 polymerase chain reaction (PCR) test for bla_{KPC} gene. If the PCR result is positive, the subject is considered not-decolonized.
- 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 versus placebo.
- Early decolonization: Negative rectal swab culture for KPC-Kp and negative polymerase chain reaction (PCR) test for *bla*KPC gene within 7-10 days of intervention.
- Persistent decolonization: Negative rectal swab culture for KPC-Kp and negative polymerase chain reaction (PCR) test for *bla*KPC gene on days 30 and 90 after the intervention.
- KPC-Kp infection: i) Proven infection: KPC-Kp isolated from clinical specimens in the presence of clinical signs and symptoms of infection; ii) 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.
- Intention-To-Treat (ITT) population: all randomized patients.

- Per protocol population (PPP): Patients who meet the following criteria: (i) having been randomized; (ii) complete data for the primary objective; (iii) not having received antibiotics between randomization and visit 3.
- Microbiologically evaluable population (PME): patients in whom all rectal colonization 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 one week before randomization.
- 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 breastfeeding.
- 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 randomization, i.e. dexamethasone, chemotherapy against solid tumors or prior to hematopoietic 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 Decolonization with oral antibiotics within 3 months prior to randomization.
- Severe food alergy.

Donor selection

General considerations

Donor selection and screening criteria for FMT is not currently standardized, 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 (**Supplementary 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 (**Supplementary 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 two months after the first donation. After this period, microbiological screening will be repeated. If the same donor is required for a new donation peirod, 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/m2).
- 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 six months, current or prior history of stay in prison, current communicable disease, risk factors for Creutzfeldt-Jakob disease, travel in the last six 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 three 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 quantitative real-time PCR.

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 (MICs) 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 (VIM) 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 quantitative real-time PCR. 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 [30,31].

Interventions

Trial intervetions

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 lyophilized 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 feces, based on previous studies supporting the efficacy of this dosing for treatment of *Clostridioides difficile* infection [32]. Participants in the placebo arm will receive 4 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 ± 3ºC, 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 one 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 decolonization 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 centralized, web-based automated randomization system, integrated with the electronic case report file (eCRF), and will be hosted by Maimonides Institute for Biomedical Research of Cordoba (Cordoba, Spain). After the patient's enrolment is confirmed, the randomization specialist will assign a computer-generated random number to each patient. The randomization 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 colonization 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 post event 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 3.1 program (https://gpower.software.informer.com/3.1/), assuming the following estimates: 90% power; 5% alpha error; decolonization rate at 30 days of 30% in the control group based on a recent metanalysis reporting CRE colonization rates of 76.7% (95% confidence interval 64%-81.8%) at 1 month in the absence of intervention [11]; decolonization rate of 60% in the experimental group, based on a recently published study [18]; 1:1 treatment to placebo ratio; superiority considered if the confidence interval lower bound for the difference between decolonization 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: a) at the request of the patient, through withdrawal of informed consent; b) when the patient no longer complies with protocol indications (protocol deviation); c) as a result of any adverse event, regardless of its intensity, at the discretion of the investigator; d) when for any reason the treatment is no longer safe for the patient; e) as a result of an administrative decision taken by the researchers, sponsor, or regulatory authority; f) 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 Chi-square or Fisher's test for categorial variables, and Student's T or Mann-Whitney U test for normally and not-normally distributed continuous variables, respectively.

The absolute difference in the percentages of decolonization between the patients in the experimental and control groups, and its 95% confidence interval, will be calculated. Clinically significant superiority will be considered if the 95% confidence interval lower bound is greater than 5%. For the primary and secondary endpoints, the main analyses will be carried out in the intention-to-treat (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 Statistics software.

ETHICS AND DISSEMINATION

The study is funded by Instituto de Salud Carlos III (Science and Innovation Ministry, Spanish government). It was authorized 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 **Annex 2**. All data will be anonymized. The study is being conducted in compliance with the protocol, regulatory requirements, International Council of Harmonization (ICH) 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. The trial is registered with ClinicalTrials.gov as NCT04760665 (February 18, 2021). 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 colonization 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 decolonization strategies.

The utility of fecal microbiota transplantation (FMT) for gut decolonization of multidrug resistant organisms has been explored in several case reports, one prospective observational cohort and one RCT, summarized 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 capsulized 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 decolonization is unclear. Firstly, the administration of antibiotics renders it very difficult to unravel the independent contributions of antibiotics and FMT to CRE decolonization. Secondly, pre-clinical studies with mouse models suggest that antibiotic pre-conditioning 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-colonized recipients [20].

Methods for FMT delivery include colonoscopy, nasoduodenal tub, colonic transendoscopic enteral tubing or oral capsules [13,41,42]. In this RCT, patients will be receiving FMT based on lyophilized oral capsules, which have been proven non-inferior to colonoscopy for the treatment of recurrent CDI and which also have higher acceptance by patients [43]. Further, patients with CRE colonization who receive oral capsulized FMT achieved high

eradication success (60%) at one month [20]. In addition, using lyophilized 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 Faecal Microbiota Transplantation 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 randomized trials have compared different amounts of faecal matter so far. In the present RCT, the capsules with the lyophilized FMT material will be provided by an external company, which has been legally authorized for production of the FMT capsules by the Spanish Agency for Medications and Healthcare Products (AEMPS). The company will guarantee that each treatment, consisting of a batch of 4 capsules, will contain a minimum of 2 x 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 decolonization to CRE eradication. If the efficacy and safety of FMT are proven, FMT may be considered a better approach for decolonization of gut MDRO than selective antibiotics decolonization, 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 [44], given the single-center nature of this RCT, they would be insufficiently represented to obtain statistically significant results that could justify their inclusion.

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CONTRIBUTIONS

JCO, EPN, JTC, AC, MR, MJA, JCP, AD, EV, CN and LMM have made substantial contributions to the design of the work, critical revision for important intellectual content, and final approval of the version to be published. JCO, EPN, JTC, AC, MR, MJA, JCP, AD, EV, CN and LMM agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

TRIAL STATUS

Current protocol approved is version 1.0 dated 4th September 2019. Recruitment will begin on 5th October 2021 and will end on 5th October 2023.

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COMPETING INTEREST STATEMENT

Juan José Castón reports personal fees from Merck for educational purposes and a research grant from Pfizer outside the submitted work. All other authors declare that they have no competing interests.

PATIENTS CONSENT FOR PUBLICATION

Not required

FIGURE LEGEND

Figure 1. Schedule of enrolment, interventions, and assessments according to SPIRIT guidelines.

Figure 1. Schedule of enrolment, interventions, and assessments according to SPIRIT guidelines.

	STUDY PERIOD						
	Enrolment	**Allocation**	**Post-allocation**				**Closeout**
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

Abbreviations: d, days; FMT, Fecal Microbiota Transplantation; $RL_{KPC,}$ relative intestinal load of bla_{KPC}.

[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.

Annex 1. SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents.

Section/item	Item No	Description	Page in the document
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	--
Protocol version	3	Date and version identifier	2~~7~~4
Funding	4	Sources and types of financial, material, and other support	2~~7~~4
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2~~7~~4
	5b	Name and contact information for the trial sponsor	--
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	2~~7~~4
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	--
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7,8
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	10-11
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	~~12-13~~14-15
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	~~14-15~~16-17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	~~12,~~ Figure 1
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	~~10-13~~13-16

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7,8, Figure 1
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	~~14-15~~16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	~~14-15~~16
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	1~~5~~3
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	1~~5~~3
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	1~~5~~3
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	1~~5~~3
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	1~~5~~3
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Figure 1
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	~~14-15~~16-17
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	~~14-15~~16-17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	~~15-16~~17-18
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	~~15-16~~17-18
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomised analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	~~15-16~~17-18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	--
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	--

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	16
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Non-applicable
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Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	14
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
	31b	Authorship eligibility guidelines and any intended use of professional writers	--
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	--
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Annex 2
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Non-applicable

Annex 1. SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents.

Section/item	Item No	Description	Page in the document
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	--
Protocol version	3	Date and version identifier	27
Funding	4	Sources and types of financial, material, and other support	27
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 27
	5b	Name and contact information for the trial sponsor	--
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Bmjopen-2021-058124_Supplemental Material

Supplemental Materials (Copyright of Mikrobiomik)

Supplementary Table S1. Donor Questionnaire

Inclusion criteria		Response
1.	Aged 18 to 50	
2.	Gender	
3.	Height	
4.	Weight	
5.	Body Mass Index *(< 30)*	

Exclusion criteria		Response
Gastrointestinal		
7.	Have you or a direct relative (parents, siblings or children) suffered from colon cancer or polyposis?	□ YES □ NOT
8.	Have you or a direct relative (parents, siblings or children) suffered from intestinal inflammatory disease (Crohn disease or ulcerative colitis?	□ YES □ NOT
9.	Do you regularly have a fever or intestinal disorders, such as diarrhoea, abdominal pain or blood in the stools?	□ YES □ NOT
10.	Do you suffer from celiac disease or other chronic digestive disorders?	□ YES □ NOT
11.	Are you diabetic?	□ YES □ NOT
Neurologic		
12.	Have you taken medications in the last 12 months, or have you been in treatment or in consultation for attention deficit or hyperactivity?	□ YES □ NOT
13.	Have you taken medication in the last 12 months, or have you been in treatment or in consultation for depression?	□ YES □ NOT
14.	In the last 12 months, have you regularly experienced symptoms of depression?	□ YES □ NOT
15.	Have you taken medications in the last 12 months, or have you been in treatment or in consultation for anxiety?	□ YES □ NOT
16.	In the last 12 months, have you regularly experienced symptoms of anxiety?	□ YES □ NOT

17.	Do you have any seasonal, food, animal, medication, latex, dust or other allergies?	□ YES □ NOT
18.	Have you had symptoms of eczema or psoriasis in the last eight weeks?	□ YES □ NOT
19.	Have you taken antibiotics, antifungals, antivirals, or any other drug that can alter the microbiota in the last three months?	□ YES □ NOT
20.	Have you taken medications related to gastric reflux?	□ YES □ NOT
21.	Have you had an asthma attack in the last 12 months?	□ YES □ NOT
22.	Have you had unprotected sex with a new partner in the last three months?	□ YES □ NOT
23.	Have you had a fever, frequent cough, or felt short of breath in the last two weeks?	□ YES □ NOT
24.	Have you gotten a new tattoo in the last six months?	□ YES □ NOT
25.	Have you had a piercing in the last six months?	□ YES □ NOT
26.	Have you been vaccinated with live attenuated virus vaccines in the last six months?	□ YES □ NOT
27.	Have you received an injection or vaccine in the last 8 weeks?	□ YES □ NOT
28.	Does your work or activity as a volunteer involve any contact with any animal or plant tissue, chronic patients, nursing homes or hospital?	□ YES □ NOT
29.	Do you have or have you ever had any type of cancer?	□ YES □ NOT
30.	If you are a woman, is there a chance you are pregnant?	□ YES □ NOT
31.	If you are a woman, Have you had a delivery or a termination of pregnancy in the last 6 months?	□ YES □ NOT
32.	What countries have you visited in the last 12 months?:	
33.	What is your highest degree of education?	
34.	Are you interested in receiving additional information?	□ YES □ NOT
35.	Reason for donating: to fight *C. difficile*, earn money, supporting research, helping patients, other.	

** Patients with affirmative responses to questions number **6, 19, 20, 23, 24, 25, 26, 27, 30, 31 and 32** (depending on the country) are classified as temporarily unrecruitable.*

** Patients with affirmative responses to questions number **7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 22, 28** y **29** are classified as definitively unrecruitable.*

Supplementary Table S2. Interview with the donor

GENERAL CRITERIA		Response
1.	Do you suffer, or have you ever suffered from any major illness (including in childhood)?	□ YES □ NOT
2.	Are you being studied for any health problems?	□ YES □ NOT
3.	Have you ever been admitted to a hospital?	□ YES □ NOT
4.	Have you ever had surgery?	□ YES □ NOT
5.	Did you receive breastfeeding in childhood?	□ YES □ NOT
6.	Have you received the vaccines correctly according to the vaccination schedule?	□ YES □ NOT
7.	Do you take any treatment regularly? If yes, please specify:	□ YES □ NOT
8.	Are you allergic to any medication or food?	□ YES □ NOT
9.	Do you drink alcohol regularly?	□ YES □ NOT
10.	Do you smoke? How many cigarettes a day?	□ YES □ NOT
11.	Have you had surgery in the last three months?	□ YES □ NOT
12.	Have you had a gastroscopy or colonoscopy in the last three months?	□ YES □ NOT
13.	Have you had a tattoo or piercing in the last six months?	□ YES □ NOT
14.	Have you been treated with acupuncture or suffered an accidental needle stick in the last six months?	□ YES □ NOT
15.	In the last 12 months, have you had contact with another person's blood?	□ YES □ NOT
16.	Have you changed your sexual partner in the last three months?	□ YES □ NOT
17.	Have you had more than one sexual partner in the last three months?	□ YES □ NOT
18.	Have you had any sexually transmitted infections in the last three months?	□ YES □ NOT
19.	Have you used any illicit drug intravenously, inhaled, snorted or by another route in the last three months?	□ YES □ NOT
20.	If you are a woman, is there any chance you are pregnant?	□ YES □ NOT
21.	If you are a woman, have you had a delivery or a termination of pregnancy in the last six months?	□ YES □ NOT
Digestive criteria		**Response**

22.	What is the frequency of your bowel movements?	
23.	What is the usual time you pass stools?	
24.	What is the usual consistency of your stools?	
25.	Do you suffer from any digestive disease?	□ YES □ NOT
26.	Are you diabetic?	□ YES □ NOT
27.	Do you suffer from irritable bowel syndrome, chronic functional constipation or chronic functional diarrhoea?	□ YES □ NOT
28.	Do you have celiac disease or any other chronic digestive disorders?	□ YES □ NOT
29.	Have you recently had diarrhoea, bloody stools, abdominal pain, or any other significant digestive symptoms in the last three months?	□ YES □ NOT
30.	Do you have a history or high risk of gastrointestinal cancer or polyposis?	□ YES □ NOT
31.	Has anyone in your family had colon cancer? (parents, siblings or children)	□ YES □ NOT
32.	Is there anyone diagnosed with Chron's disease or ulcerative colitis in your family? (parents, siblings or children)	□ YES □ NOT
33.	Have you undergone major surgery on the digestive system? (excluding appendicectomy) (parents, siblings or children)	□ YES □ NOT
Infectious Diseases Criteria		**Response**
34.	Have you or someone close to you suffered from a COVID-19 infection?	□ YES □ NOT
35.	Have you suffered from malaria, Chagas disease or babesiosis?	□ YES □ NOT
36.	Have you ever had a positive test for HIV?	□ YES □ NOT
37.	Have you ever had HTLV (human T-cell lymphotropic virus type 1 and 2) or tuberculosis?	□ YES □ NOT
38.	Have you had risky sexual relations (i.e. sexual contact with strangers, prostitutes, drug addicts, patients with HIV, patients with viral hepatitis, syphilis or have you worked as a prostitute?	□ YES □ NOT
39.	In the last 12 months, have you had sexual contact with someone who used needles for drugs, steroids or anything else that a doctor did not prescribe?	□ YES □ NOT
40.	In the last 12 months, have you had or been treated for any sexually transmitted disease? (syphilis, gonorrhoea)	□ YES □ NOT
41.	Have you had sexual contact with someone with HIV, hepatitis B or hepatitis C?	□ YES □ NOT
42.	Have you been in contact with another person's blood or been pricked with any Sharp material that could be contaminated with another person's blood or fluids in the last six months?	□ YES □ NOT

43.	In the last 12 months, have you had sexual contact with someone suffering from haemophilia or receiving clotting factor concentrates?	□ YES □ NOT
44.	If you are a female donor, in the last 12 months, have you had sexual contact with a man who has ever had sexual contact with another man?	□ YES □ NOT
45.	If you are a male donor, have you ever had sexual contact with another man?	□ YES □ NOT
46.	Have you had any recent infections by gastrointestinal microorganisms?	□ YES □ NOT
47.	Have you been outside of Spain in the last three years? Discuss your trips and activities with your doctor.	□ YES □ NOT
48.	Have you ever spent more than a month in any country in Latin America, Asia or Africa?	□ YES □ NOT
49.	Have you travelled in the last six months to tropical countries with endemic diarrheal diseases or those with a risk of traveller's diarrhoea?	□ YES □ NOT
50.	Between 1980 and 1996, were you in the UK for more than three months?	□ YES □ NOT
51.	From 1980 to the present, have you received a blood transfusion in the UK or France?	□ YES □ NOT
53.	Have you had contact with someone vaccinated for smallpox in the last eight weeks?	□ YES □ NOT
54.	Have you been vaccinated with live attenuated virus vaccines in the last six months?	□ YES □ NOT
55.	Have you had an injection or vaccine in the last eight weeks?	□ YES □ NOT
Others		**Response**
56.	Do you have cancer, or have you had it in the last ten years?	□ YES □ NOT
57.	Do you suffer from any blood disease or any tendency to bleed?	□ YES □ NOT
58.	Have you received any transfusion of blood or derived products in the last 12 months?	□ YES □ NOT
59.	Have you received a tissue (bone or skin), organ, or bone marrow graft in the last 12 months?	□ YES □ NOT
60.	Have you ever had a dura mater graft or brain sheath graft?	□ YES □ NOT
61.	In the last 16 weeks, have you donated red blood cells through an apheresis machine?	□ YES □ NOT
62.	Do any of your relatives have Creutzfeld-Jakob disease?	□ YES □ NOT
63.	In the last 12 months, have you been in a correctional or correctional facility or arrested for more than 72 hours?	□ YES □ NOT
64.	Are you a health worker or a worker in a hospital or health institution?	□ YES □ NOT
65.	Do you work with animals?	□ YES □ NOT

66.	Do you have the legal capacity to sign informed consent?	□ YES □ NOT
67.	Have you ever had any heart or liver problems?	□ YES □ NOT
68.	Do you have chronic hepatitis?	□ YES □ NOT
69.	Do you suffer from chronic renal insufficiency?	□ YES □ NOT
70.	Do you suffer from autoimmune diseases affecting the digestive tube?	□ YES □ NOT
71.	Do you suffer from metabolic syndrome?	□ YES □ NOT
72.	Do you suffer from any neurological, neurodegenerative or psychiatric disease?	□ YES □ NOT
73.	Do you suffer from vascular disease?	□ YES □ NOT
Drugs		**Response**
74.	Have you taken antibiotics, antifungals, antivirals, or any other drug that alters the microbiota in the last three months?	□ YES □ NOT
75.	Have you taken proton pump inhibitors in the last three months?	□ YES □ NOT
76.	Have you received immunosuppressive medication or chemotherapy in the last three months?	□ YES □ NOT
77.	Have you received systemic antineoplastic agents in the last three months?	□ YES □ NOT

Supplementary Table S3. Microbiological screening for donors

1. DONOR BLOOD SCREENING		
1.1. GENERAL LABORATORY		
1.1.1.	Hemogram ☐	
1.1.2.	Biochemistry: ☐ Creatinine ☐ Urea ☐ Glucose ☐ Sodium ☐ Potassium ☐ Chloride ☐ Calcium ☐ Magnesium	☐ Phosphorus ☐ Uric acid ☐ Alanine aminotransferase ☐ Aspartate aminotransferase ☐ Alkaline phosphatase ☐ Total bilirubin ☐ Albumin ☐ C-reactive protein
1.1.1.	Lipids: ☐ Triglycerides ☐ Total cholesterol ☐ High-density lipoprotein ☐ Low-density lipoprotein	
1.2. MICROBIOLOGICAL STUDIES		
	Hepatitis A virus:	☐ Immunoglobulin M (IgM) ☐ Immunoglobulin G (IgG)
	Hepatitis B virus:	☐ Serum hepatitis B surface antigen ☐ Antibodies to hepatitis B core antigen (IgG) ☐ Antibodies to hepatitis B core antigen (IgM) ☐ Hepatitis B surface antibody
	Hepatitis C virus:	☐ Hepatitis C immunoglobulin
	Hepatitis E virus:	☐ Ig M

☐ Ig G
Human immunodeficiency virus: ☐ HIV-1/HIV-2 antibodies/p24 test
SARS-COV-2: ☐ Ig M ☐ Ig G
Syphilis: ☐ Rapid plasma reagin *(If reactive, a FTA-ABS test will be performed)*
Cytomegalovirus: ☐ IgM ☐ IgG
Epstein-Barr virus: ☐ IgM ☐ IgG
2. SCREENING OF DONOR FECES
☐ *Clostridiodes difficile*: Glutamate dehydrogenase (GDH9 testing assay and/or toxin A and B. *(if GDH is positive, a test for toxins A and B or culture will be performed*
☐ *Giardia Lamblia* antigen test
☐ Helicobacter Pylori antigen test
☐ Strongyloides
☐ *Giardia lamblia*
☐ *Salmonella* spp.
☐ *Shigella* spp.
☐ *Campylobacter* spp.
☐ Enteropathogenic *Escherichia coli*
☐ *Yersinia* spp.
☐ *Vibrio cholerae*
☐ *Listeria monocytogenes*
☐ Blastocystis
☐ *Entamoeba histolytica*

☐ *Cryptosporidium*
☐ Norovirus
☐ Adenovirus
☐ Rotavirus
☐ Ova and parasite test
Multidrug-resistant bacteria: ☐ Extended-spectrum beta-lactamase-producing *Enterobacterales* ☐ Carbapenemase-producing *Enterobacterales* ☐ Vancomycin-resistant *Enterococci* ☐ Methicillin-resistant *Staphylococcus aureus*
☐ Fecal occult blood test
☐ Fecal calprotectin
3. **NASOPHARINGEAL SCREENING FOR SARS-CoV2/COVID-19**