CLINICAL RESEARCH PROTOCOL

Open-label, randomized study to assess the efficacy of a probiotic or fecal microbiota transplantation (FMT) on the eradication of rectal multidrug-resistant Gram-negative bacilli (MDR-GNB) carriage

Protocol: PROFTMDECOL
Version: 1.0 (02/12/2019)

Sponsor: Fundació Clínic per a la Recerca Biomèdica

Principal Investigator: Dra. Ana Del Río.
Co-principal Investigator: Dr. José Antonio Martínez.
Infectious Diseases
Hospital Clínic
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1. Brief description

1.0. Study

This is an investigator-initiated, single center, parallel-group, randomized, open-label, clinical research with non pharmacological products.

1.1. Sponsor

Fundació Clínic per a la Recerca Biomédica.
Rosselló, 149
08036 Barcelona

1.2. Study identification

Title: Open-label, randomized study to assess the efficacy of a probiotic or fecal microbiota transplantation (FMT) on the eradication of rectal multidrug-resistant Gram-negative bacilli (MDR-GNB) carriage.

Protocol code: PROFTMDECOL

1.4. Investigators

Principal investigator: Dra. Ana Del Río, Infectious Diseases
Co-principal investigator: Dr. José Antonio Martínez, Infectious Diseases

Collaborators:

Dr. C. Pitart, Microbiología Clínica
Dr. J. Mercadal, Anestesiología
Dra. E. López, Farmacia Hospitalaria

1.5. Clinical sites

Single centre study: Hospital Clínic, Barcelona

1.6. Ethics approval

The protocol, informed consent form, and other required documents will be approved by the Ethics Committee (Comité de Ética de la Investigación, CEI) of the Hospital Clínic de Barcelona before enrolment of subjects in the study.

1.7. Monitoring

Dr. JA Arnaiz, Unidad de Ensayos Clínicos (CTU Clinic)
Farmacología Clínica, Hospital Clínic, Barcelona.

1.8. Treatments

Eligible subjects will be randomly assigned to one of the two intervention arms (probiotic or fecal microbiota transplantation product) or to a control non-intervention (observation) arm in a 2:2:1 proportion.

a) Probiotic (Vivomixx® 2 sachets/12h for 2 weeks)
b) Fecal microbiota transplantation: administration of two doses (administered a week apart) of a fecal microbiota transplantation preparation (14-17 oral capsules per dose equivalent to 50 g of healthy-donor feces)
c) No treatment (Observation)

1.9. Primary objective

To compare the decolonization efficacy at the end of the study (60 ± 7 days after randomization)

1.10. Study population

Patients with rectal colonization with MDR-GNB (ESBL-producing *Klebsiella pneumoniae,* CPE and MDR/XDR *Pseudomonas aeruginosa*).

1.11. Subject selection

Inclusion criteria

Male and female patients who meet all the following criteria will be eligible for this study:

1. Adult patients (≥18 years-old).
2. Admitted to the Hospital Clinic of Barcelona with documented rectal colonization whitin the previous 7 days by rectal swabbing with MDR-GNB (ESBL-producing *Klebsiella pneumoniae,* carbapenemase-producing Enterobacterial (CPE) and MDR/XDR *Pseudomonas aeruginosa*).
3. Eligible for routine digestive decolonization (7 days oral administration of nonabsorbable antibiotics (NAA).
4. Capable to provide informed consent (by themselves or through their legal representatives).

Exclusion criteria

Patients meeting any of the following criteria will not be eligible for the study:

1. Pregnant women or breastfeeding.
2. Neutropenic patients (total neutrophil count <500 cell/mm^3)*.
3. HIV-infected patients with CD4 count <200 cell/mm^3.
4. Patients with active *C. difficile* infection.
5. Patients with ileus or bowel obstruction.
6. Patients with documented or suspected bowel perforation.
7. Patients with a colistin-resistant MDR-GNB.

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1.12. Assessment

Decolonization rate at the end of the study (60 ± 7 days after randomization**).** Decolonization defined as a negative rectal swab sample for the target MDR-GNB at 60 ± 7 days after randomization**.**

1.13. Allocation participant

A total of 437 eligible subjects will be randomly assigned to one of the two intervention arms (175 patients to probiotic, 175 to fecal microbiota transplantation product) or to a control non-intervention arm (87 patients). Randomization will be stratified by the type of microorganism (ESBL-*K. pneumoniae*, CPE and MDR-*P. aeruginosa*) to ensure a balanced number of each organism in the study arms.

1.14. Follow-up

Patients follow-up will be carried out 60 days (± 7 days) after randomization.

1.15. End of the clinical research

The end of the clinical research is defined as the date of the last visit of last subject undergoing the study with a recruiment period of 30 months.

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3. GENERAL INFORMATION

3.1. Study identification

Title: Open-label, randomized study to assess the efficacy of a probiotic or fecal microbiota transplantation (FMT) on the eradication of rectal multidrug-resistant Gram-negative bacilli (MDR-GNB) carriage.

Protocol code: PROFTMDECOL

3.2. Investigators

Principal investigator: Dra. Ana Del Río, Infectious Diseases
Co-principal investigator: Dr. José Antonio Martínez, Infectious Diseases

Collaborators:

Dr. C. Pitart, Clinical Microbiology
Dr. J. Mercadal, Anaesthesiology
Dra. E. López, Hospital Pharmacy

3.3. Clinical sites

Single centre study: Hospital Clínic, Barcelona

3.4. Sponsor

Fundació Clínic per a la Recerca Biomédica.
Rosselló, 149
08036 Barcelona

3.5. Monitoring

Clinical Trials Unit (CTU Clinic)
Clínical Pharmacology
Hospital Clínic, Barcelona.

3.6. Study duration

Once approval is obtained, by January 2020, recruitment period will last 30 months.
Patient follow-up will be 60+/- 7 days for each patient.
End of study corresponds to last patient-last visit and is expected by September 2022.
The analysis of the results and the study final report will be finalised by December 2022.

4. BACKGROUND, RATIONALE AND OBJECTIVES

4.1. Background and rationale

The extension of **multidrug-resistant gram-negative bacilli** (MDR-GNB) in health-care centers is a growing problem worldwide, with economic implications that have a negative impact on the efficiency of health systems. The WHO considers antibiotic resistance as "a growing threat to global public health that requires coordinated action by governments and society." In our hospital, the consumption of active antibiotics against MDR-GNB increased in 2017 with respect to the previous year by 20% in the case of meropenem and ceftazidime-avibactam and in 140% for ceftolozane-tazobactam. The most frequent and clinically relevant multidrug-resistant bacteria isolated in patients admitted to our center are extended-spectrum beta-lactamase- and/or carbapenemase-producing (ESBL) *Klebsiella pneumoniae*, other carbapenemase-producing Enterobacterial (CPE) and MDR/XDR *Pseudomonas aeruginosa*. We will refer to all of them as multidrug-resistant gram-negative bacilli (MDR-GNB).

The **rectal carrier** status of MDR-GNB is a risk factor for developing an infection (bacteremia, urinary infection, peritonitis, pneumonia) by these microorganisms. The percentage of colonized patients who end up developing an infection ranges between 4% and 20%, although this percentage can reach 50% in certain populations such as patients admitted to the ICU, those who undergo a transplant of hematopoietic precursors, hepatic graft recipients or those submitted to an intra-abdominal surgical procedure (1-4). The crude mortality of these patients is around 40%, which is due, at least in part, to the fact that in most cases the therapeutic alternatives are less effective and more toxic (colistin, amikacin, tigecycline). These data highlight the need of establishing strategies to prevent the transmission of MDR-GNB on one hand and of eradicating or reducing carriage in order to improve the prognosis of colonized patients on the other.
The most effective measures to prevent horizontal patient-to-patient transmission of MDR-GNB include: 1) hand hygiene, as the most effective isolated measure 2) cleaning and disinfection of surfaces, 3) application of contact precautions (isolation) to colonized patients and 4) decolonization.

Decolonization is the only measure designed to improve the prognosis of the patient who is already colonized, but there is no consensus on its widespread use mainly due to the fear of selecting panresistant strains (resistant to all available antibiotics) (5). Without any therapeutic intervention, the rate of spontaneous decolonization is low, particularly among the most fragile patients (tracheal intubation, decubitus ulcers, multiple vascular catheters, nasogastric tube) and those requiring prolonged antibiotic treatment (6,7). In this population, a retrospective study conducted in a hospital in Spain showed that the decolonization of patients with MDR-GNB with oral gentamicin significantly reduced mortality, but in up to 15% of patients gentamicin resistance emerged (8). These results support the need of evaluating new decolonization strategies that minimize the resistance selection risk. For digestive decolonization, oral administration of nonabsorbable antibiotics (NAA) such as colisitin or aminoglycosides has been the most common approach. Administered in this way they are non-toxic and reach high concentrations in feces. A recent systematic review described an eradication rate at the end of treatment (7-14 days) of 65%, but at one month of follow-up the rate was reduced to 40% (9). In addition, among patients with CPE, resistance to colistin or gentimycin has been detected in 8% and 45% of those who received monotherapy with these antibiotics, respectively (10-12). In contrast, a prior study showed that resistance was not selected in any of the 5 patients who received the combination of both antibiotics (11). In this regards, a regimen consisting of 100 mg of colistin together with 80 mg of tobramycin and 500 mg of amphotericin B 4 times a day has been shown to be beneficial in critically ventilated patients both in terms of reducing mortality and reducing the incidence of bacteremia (13,14).

In critically ill patients treated with this regimen, one study showed that the acquisition of colistin-resistant enterobacteria was 5 times higher in those previously colonized by strains resistant to tobramycin and therefore receiving monotherapy with colistin (15). In countries with a low incidence of resistance, intestinal decontamination with colistin-tobramycin has been associated with an increase in resistance to tobramycin, although its overall prevalence has remained below 1% and the efficacy of the procedure has not been compromised (16). Finally, in a recent randomized study on ventilated patients in ICUs with moderate to high prevalence of antibiotic resistance, although neither oral nor digestive decontamination with colistin-tobramycin was effective for reducing ICU-acquired bacteremia due to MDR-GNB, the prevalence of colistin resistance did not increase during the intervention periods (17).
In our environment, the prevalence of resistance to gentamicin and tobramycin is high among the ESBL-producing enterobacteria (22-54% in E. coli and 31-67% in K. pneumoniae) and CPE (67%-74%). Amikacin maintains its activity against more than 90% of ESBL-producing enterobacteria and ≥80% of CPE, making it the ideal aminoglycoside to accompany colistin. The latter is still active against >95% of the strains of interest. Other antibiotic options have hardly been explored and, of these, rifaximin is probably one of the most interesting. Rifaximin is a non-absorbable rifamycin used for the treatment of traveler's diarrhea, hepatic encephalopathy, and other gastrointestinal disorders (18). Its fecal concentration after taking 400 mg/12 h for 3 days is around 8000 mg/L and has a good activity against strains of *E. coli* producing ESBL and several CPE. In our experience in 54 patients of an hepatic ICU colonized by MDR-GNB during 2016-2017, the pattern of decolonization with a poly-antibiotic solution constituted by colistin (10 mg/mL), amikacin (8 mg/mL) and amphotericin B (50 mg/ml) 10 mL every 6 h for 10 days (in patients with strains sensitive to colistin and amikacin) or 10 mL of poly-antibiotic solution every 6 h plus rifaximin 400 mg/8 h for 10 days (in patients with colistin-resistant strains or amikacin) was 50% at one month of follow-up (19). This antibiotic regimen is used routinely for the decolonization of rectal carriers of MDR-GNB in our center.

Probiotics are a combination of different live microorganisms that administered orally produce qualitative changes in the intestinal microbiota that have been related to an efficient protection against *Clostridium difficile*. They are considered dietary supplements for administrative/legal purposes. There is some published experience with probiotics in the prevention of infections in hospitalized patients, but additional clinical trials are necessary to support its use. Regarding current published experience, the combination of three Lactobacillus (*L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2) was administered to all patients admitted to a Quebec hospital and receiving an antibiotic (20). After 10 years of experience (44,835 patients included), the incidence of *C. difficile* decreased from 18/10,000 patient-days to 2.3/10,000 patient-days. On the other hand, it has been observed that patients acquiring a MDR-BGN during admission have a lower percentage of *Lactobacillus spp*. in the intestinal flora than those who do not become colonized and that *Lactobacillus delbrueckii* subspecies delbrueckii LDD01 (DSM22106) inhibits in vitro the growth of *Klebsiella spp.* probably through the production of a bacteriocin (21,22). Based on the above, it seems reasonable to hypothesize that certain probiotics can compete for adhesion to the intestinal mucosa and nutrients with other microorganisms of the intestinal flora and consequently favor the decolonization of MDR-GNB. In addition, as probiotics are almost free of safety concerns or resistance selection issues, they are a therapeutic option that requires evaluation.
The probiotic that we propose to use in this study is Vivomixx® powder in sachets, composed of 8 living bacterial species (*L. paracasei* DSM 24733, *L. acidophilus* DSM 24735, *L. delbrueckii ssp bulgaricus* DSM 24734, *L. plantarum* DSM 24730, B. brief DSM 24732, *Bifidobacterium longum* DSM 24736, B*. infantis* DSM 24737, *Streptococcus thermophilus* DSM 24731) with a concentration of 450 billion live lyophilized bacteria per sachet. There are numerous studies on the use of Vivomix® in different disorders, particulary to prevent hepatic encephalopaty in cirrhotic patients and in patients with inflammatory bowel disease where it has been shown to be very safe. In our opinion, the lack of reports of bacteremia associated with Vivomix®

consumption in patients with liver cirrosis, a population at high risk of intestinal bacterial translocation, and the rarity of serious adverse effects associated with bacterial probiotics in general in other sensible population such as the critically-ill or liver transplant recipients is enough evidence to consider the product as probably safe for the main target population of the present study (23-28).

Regarding the use of probiotics for the decolonization of MDR-GNB, clinical evidence is limited to three trials. A randomized study in 103 ICU patients published in 2015 did not found significant differences in acquisition or loss of MDR organisms, including *Pseudomonas aeruginosa* and ESBL- or carbapenemase-producing Enterobacterials between patients who received *L. rhamnosus* GG (1 capsule containing 1010 CFU every 12h for 14 days) and those not receiving the probiotic (29). In a more recently published randomized trial, the administration of a symbiotic (1010 CFU *L. bulgaricus* plus 1010 CFU *L. rhamnosus* plus fructo-oligosaccharides twice a day for 1 week) to 101 evaluable hospitalized patients colonized by MDR-GNB was not associated with improved rates de decolonization or infection (30). It is of note that in both studies the number of patients was small and the probiotic consisted in one or two bacterial species. Lastly, in a small (80 patients) randomized comparative trial, although Vivomixx® was associated with a 2.7 higher rate of intestinal eradication of ESBL-producing enteric gram-negative bacilli (mostly *E. coli*) than placebo, the total proportion of decolonized patients whitin one year was small (12.5%) and not significatively different from placebo (31). However, our group has used Vivomixx® associated with NAA for the decolonization of patients in a recent outbreak of KPC-producing *Klebsiella pneumoniae*. The rate of decolonization at one month after diagnosis in patients who received NAA followed by Vivomixx® during 20 days was higher than that of those who received only NAA (88% vs. 68% p = 0.008). These results have been accepted as an oral communication at the 29th European Congress of Infections (ECCMID) (Amsterdam April 13-16, 2019) (19). We think that a rigurous further clinical evaluation of probiotics as a decolonization tool in rectal carriers of MDR-GNB is justified.

Faecal microbiota transplantation (FMT), a highly effective treatment for recurrent *Clostridium difficile* infection, is a promising therapy for the intestinal decolonization of MDR-GNB. Some individual cases or small series have been published in which FMT has been effective for the intestinal decolonization of ESBL- and CPE, carbapenemase-producing *A. baumannii*, vancomycin-resistant enterococci or methicillin-resistant *Staphylococcus aureus* (32-38). The longest prospective series published so far includes 20 cases of intestinal colonization with MDR-GNB in hematological patients (39). Complete decolonization at one month was achieved in 60% and at 6 months in 93%. In the case of *Klebsiella pneumoniae* (the most frequent in our center) the percentage of decolonization at one month was 53% (60% for NDM1-producing strains). The results of a small multicentre randomized open-label trial comparing 5 days of NAA followed by FMT versus no treatment has been recently published by Huttner BD et al (40). This study included 39 rectal carriers of ESBL- or CPE, of whom 22 received FMT administered via frozen capsules or a nasogastric tube. There were no statistically significant differences in the rate of decolonization between the two groups at 35-48 days after randomization, although a higher proportion of patients in the intervention arm was decolonized (41% vs. 29%). In this study, it is of note that a rebound in the proportion of patients with a positive stool culture was observed within the two weeks after FMT. In a retrospective matched case-control study by Saidani N. et al. (41) analysing 10 carriers of CPE treated with a 5-day course of NAA followed by FMT administered via nasogastric tube, 80% of patients had a negative rectal swap 14 days after FMT but 5 of the 10 patients received 2 FMT, suggesting that in order to achive a timely high rate of decolonization, a single FMT may not be enough. Currently, the role of FMT for erradication of MDR organisms rectal carriage is unclear and many questions remain to be answered, among them which are the most effective raw material (from pooled or single donors), preparation (fresh, frozen, lyophilized or others), dosage and route of administration, if there are specific caracteristics of the donor flora associated with decolonization, or if there are important particularities in the entire FMT protocol (NAA quality, dose and duration; prior mechanical cleaning of the colon; detection and

decolonization of other potentially colonized sites). It seems clear from what has been published so far that the decolonizing effectiveness of FMT decreases significantly when the patient also receives systemic antibiotics and that the procedure works and is safe even in immunosuppressed patients. Currently, several clinical trials are underway to investigate the decolonization efficacy of FMT on MDR organisms that may help clarify some of these aspects. To date, no consensus has been reached on the cataloging of FMT for administrative-legal purposes, which is why it is not currently considered a medicine.
Our group received financing from FIS (Stability and viability of liophylized intestinal microbiota for the treatment of *Clostridium difficile* infections. Fondo de Investigación Sanitaria (FIS) PI16/01023. Period: 2017-2019. Principal Investigator: Alex Soriano Viladomiu) to develop a method of processing feces to do fecal transplant in patients with *C.difficile* infection. It has been achieved to process the samples of healthy volunteers to obtain adsorbate capsules that can be stored at 4ºC. Furthermore, the project includes a pilot study in which patients treated for *C.difficile* will receive a dose of these capsules to prevent infection recurrence. The absorbate capsules are the FMT preparation intended to be used in the present clinical research. A brief description of the procedure to obtain them is provided in section 7 "Description of study treatments".

In summary, this project will explore the role of probiotics and FMT in eradicating the rectal carriage of MDR-GNB, aimed at addressing the growing problem of multiresistance in hospitals, which is one of most important challenges that the health system currently has to face.

Hypothesis:

The working hypothesis is that in patients who are rectal carriers of MDR-GNB, the rate and speed of erradication of the carrier status obtained with NAA regimens are insufficient and could be improved with additional interventions directed to restore a healthy fecal microbiota or to increase the colonization resistance by the putative beneficial activity of lactate-producing bacteria and bifidobacteria. A healthier colonic microbiota environment is expected not only to promote the erradication of the existing MDR organisms but also to hinder the subsequent recolonization and hopefully the risk of infection with gut dysbosis-associated pathogens (MDR-GNB, *C. difficile* and *Candida*).

Obtaining decolonization rates over 75% in any of the study arms will be of potential clinical relevance.

4.2. Objectives of the study

4.2.1. Primary objective

To compare the decolonization efficacy at the end of the study (60 ± 7 days after randomization) of the administration of a probiotic (Vivomixx® 2 sachets/12h for 2 weeks) versus the administration of two doses (administered a week apart) of a fecal microbiota transplantation preparation (14-17 oral capsules per dose equivalent to 50 g of healthy-donor feces) and no treatment (control arm) in patients with rectal colonization with MDR-GNB (ESBL-producing *Klebsiella pneumoniae*, CPE and MDR/XDR *Pseudomonas aeruginosa*).

4.2.2. Secondary objectives

1.- To determine the rate of digestive decolonization (negative fecal sample for the target MDR-GNB) at the end of the decolonization treatment (week 3 after randomization).
2. - To determine the efficacy of the decontamination with NAA defined as the rate of digestive decolonization (negative rectal swab [RS]) for the target MDR-GNB at the end of

the decontamination period (week 1 after randomization).
3.- To determine the incidence of patients with positive clinical samples for either the same microorganism colonizing their digestive tract or any other MDR-GNB during the study period.
4.- To determine the incidence of clinical infections (including those due to *C. difficile*) during the study period.
5.- To determine the prevalence of resistance emergence to any of the antibiotic components of the NAA regimen (colistin, amikacin) in those patients with persistently positive control RS or fecal sample.
6.- To assess the fecal microbiota at baseline and at the end of treatment (week 3 after randomization) in order to evaluate the degree of fecal microbiota restoration, engrafment success and relationship of fecal composition with MDR-GNB eradication.

5. TYPE OF STUDY AND DESIGN

5.1. Study design

This is an investigator-initiated, single center, parallel-group, randomized, open-label, clinical research with non pharmacological products.

Participants will receive treatment during two weeks. Each patient will be followed for a period of 60 days. Six study visits will be carried out during this period: screening, baseline, treatment intiation visit, treatment follow-up visit, end of treatment visit and end of study visit.

Limitations of the study: The main limitation of the study is a moderate sample size that may raise power questions regarding comparisons stratified by bacterial species.

5.2. Criteria for termination and /or discontinuation

The participants will discontinue study participation if they are unwilling or unable to meet the protocol requirements in terms of the visit schedule or if the patient or the investigator considers it is best to end their participation in the study. All participants have the right to withdraw their consent at any time during the study without prejudice to them.
All follow-up terminations of study subjects and the reasons for them must be reported immediately to the study monitor and be duly documented both in the medical records and the case report form.

5.3. Drug accountability

Drug accountability will be carried out at each study visit for those patients assigned to the Probiotic arm.
FMT administration will always be performed under direct observation of one of the investigators and if ambulatory, the patient will remain under observation in the outpatient clinic during at least 2 hours after the procedure.

5.4. Allocation participant identification code

Eligible subjects will be randomly assigned to one of the two intervention arms (probiotic or fecal microbiota transplantation product) or to a control non-intervention (observation) arm in a 2:2:1 proportion. Randomization will be stratified by the type of microorganism (ESBL-*K. pneumoniae*, CPE and MDR-*P. aeruginosa*) to ensure a balanced number of each organism in the study arms.

Randomization list will be computer generated. The investigators will not be aware of the patient assignment until the inclusion.

5.5. Source data verification

Source documents are defined as all observations or notes recorded on the clinical interventions, and all reports and notes required for assessment and reconstruction of the research study.
Whenever possible the original document should be kept as the source document; however, provision of a photocopy which is clear, legible and an exact duplicate of the original document and signed by the principal investigator is acceptable.

5.6. End of the clinical research

The end of the clinical research is defined as the date of the last visit of last subject undergoing the study.

6. SUBJECT SELECTION

6.1 Inclusion criteria

Male and female patients who meet all the following criteria will be eligible for this study:

5. Adult patients (≥18 years-old).
6. Admitted to the Hospital Clinic of Barcelona with documented rectal colonization whitin the previous 7 days by rectal swabbing with MDR-GNB (ESBL-producing *Klebsiella pneumoniae*, carbapenemase-producing Enterobacterial (CPE) and MDR/XDR *Pseudomonas aeruginosa*).
7. Eligible for routine digestive decolonization (7 days oral administration of nonabsorbable antibiotics (NAA).
8. Capable to provide informed consent (by themselves or through their legal representatives).

6.2 Exclusion criteria

Patients meeting any of the following criteria will not be eligible for the study:

8. Pregnant women or breastfeeding.
9. Neutropenic patients (total neutrophil count <500 cell/mm^3)*.
10. HIV-infected patients with CD4 count <200 cell/mm^3.
11. Patients with active *C. difficile* infection.
12. Patients with ileus or bowel obstruction.
13. Patients with documented or suspected bowel perforation.
14. Patients with a colistin-resistant MDR-GNB.

* Solid organ or haematopoietic organ transplant recipients without neutropenia will qualify for inclusion in the study. This is based on preliminary data on the safety of probiotics without *Sacharomyces boulardii/cerevisae* in transplant recipients of several organs (hematopoietic precursors, liver, kidney, small intestine) and on the preliminary safety data of FMT in non-neutropenic patients with hematological disorders including hemotopoietic cell transplantation (25,42,43). However, the condition of transplant recipients will be assessed in an individual basis and only those patients that by consensus with their attending physician

will be judged to be at a very low risk of complications derived from the use of the probiotic o fecal microbiota will be considered for inclusion in the study.

6.3. Enrolment period

In accordance with prior local data, we estimate that recruitment of 437 subjects will require 30 months.

6.4. Patient withdrawal and study discontinuation

A patient will prematurely discontinue the study in case of withdrawal of informed consent.
All patients have the right to withdraw their consent at any time during the study without prejudice to them.
In addition, the investigator may decide, for reasons of medical prudence, to remove the patient from the study.
The date and reasons for stopping the study will be clearly stated on the subject's CRF and source document.

7. DESCRIPTION OF STUDY TREATMENTS

7.1 Study arms

The eligible subjects will be randomly assigned to receive, after a 7-day decolonization course of oral non-absorbable antibiotics (NAA), as standard of care, to one of the following regimens:

a) Probiotic regimen

Participants assigned to the probiotic arm will receive 2 sachets of Vivomixx® dissolved in 50 L of warm water (according to the manufacturer indications) every 12 h orally or through a nasogastric tube (in this case the tube will be flushed with 50 mL of water after the administration). The probiotic will be started 24 h after the last dose of NAA and will be administered for 14 consecutive days. Each sachet of Vivomixx® contains a combination of 4 Lactobacillus (*L. paracasei* DSM 24733, *L. acidophilus* DSM 24735, *L. delbrueckii ssp bulgaricus* DSM 24734, *L. plantarum* DSM 24730), 3 Bifidobacteria (*B. brief* DSM 24732, *B. longum* DSM 24736, *B. infantis* DSM 24737), and *Streptococcus thermophilus* DSM 24731 at a concentration of 450 billion ($45x10^{10}$) live lyophilized bacteria per sachet.

b) FMT regimen

The FMT preparation will be administered as 2 doses, once a week, of 14-17 capsules per dose. Each dose (14-17 capsules) will contain the fecal microbiota equivalent to 50 gr of stools from a healthy donor. If the patient is carrying a nasogastric tube, the content of capsules will be decapsulized (~27 gr of powder), diluted in 50 mL of water and passed thorugh it, followed by the administration of 50 mL of water to flush the tube. The first dose will be administered 24 h after the last dose of NAA and the second dose one week later. Whenever possible, the two doses of FMT will be originated from the same donor. According to our data done by flow cytometry and bacterial culture in three replicates, each absorbate capsule contains approximately $1.12x10^{10}$ live bacteria.

c) Control arm

Patients assigned to the control arm (observation arm) will not receive other treatments beyond the standard 7-day course of NAA.

7.2 Dose selection

The most effective dose of the probiotic is unknown, hence the dosage of Vivomixx® has been selected to ensure an exposure to probiotic bacterial cells not inferior to that of the FTM produt while still being safe as proven in a prior clinical trial (31).

The most efective dosage of FMT is also unclear. The one selected in the present study correspond to the most effective dosing strategy for the treatment of recurrent *C. difficile* colitis (44).

7.3. Product information

Vivomixx®

Vivomixx® is a dietary supplement. A 4.4g sackett contains 450 billions of live lactobacilli and bifidobacteria (*Streptococcus thermophilus* DSM24731®, *Bifidobacterium breve* DSM24732®, *Bifidobacterium longum* DSM24736®, *Bifidobacterium infantis* DSM24737®, *Lactobacillus acidophilus* DSM24735®, *Lactobacillus plantarum* DSM24730®, *Lactobacillus paracasei* DSM24733®, *Lactobacillus delbrueckii ssp. bulgaricus* DSM24734®), maltose, anti-caking agent: silicon dioxide.

Vivomix® will be provided by the sponsor of the study. It will be administered using the common procedures of the clinical practice in the participating site. Patients will receive the marketed product.

Source of FMT:

Obtaining adsorbate capsules from feces

The fecal mibrobiota preparation will be provided by the sponsor of the study.
The obtention, processing feces and capsules preparation to do the fecal transplant has been already developed in our center.
Healthy donors are selected after screening composed by a form, an interview and blood and fecal analysis. The stool sample is collected in a plastic recipient (Fecotainer®, AT Medical B.V., Netherlands) with BD Gaspak™ EZ anaerobe system (Becton Dickinson and Company, USA) inside to reach anaerobiosis, and it is received in the laboratory within 4-6 hours of collection.

Each sample is separately labelled, weighed and transferred to a stomacher classic bag. Sterile saline is added in 10:1 proportion and the mix is homogenated in Stomacher 400 circulator (Seward Ltd., Sussex, United Kingdom) for 1 minute at 230rpm. Then, the slurry is transferred to a falcon tubes and 10% of Glycerol at 99% is added to the solution with a motorized pipette.

The samples are frozen at -80ºC until its use. When required, samples are thawed overnight at 4ºC and 20% of Glycerol at 99% is added. They are centrifuged in Heraeus Megafuge 16R Centrifuge (Thermo Fisher Scientific Inc., MA, USA) for 20 minutes at 400g, 4ºC with slow deceleration to remove sample debris. Then, the supernatant is transferred into high resistant tubes previously filtered with conventional sieve to eliminate possible detritus and the volume is centrifuged for 30 minutes at 10000g and 4ºC (Sorvall Evolution RC Centrifuge, Thermo Fisher Scientific Inc., MA, USA). We eliminate the supernatant leaving pellet, where we found the bacteria. The volume of pellet equivalent to 50g of feces is mixed manually in a mortar with Vivapur-101® (JRS Pharma) and

Magnesium stearate until achieve a homogenous powder obtaining an adsorbate product. The mix is encapsulated with semi-automated encapsulator FagronLAB™ FG (Fagron Iberica, Barcelona, Spain) into acid-resistant capsule size nº00.

Around 14-17 capsules are obtained from 50g of faeces that are kept at 4ºC with Silica Gel labelled for its traceability until its use. We have tested the viability and quantity of bacteria in these capsules by flow cytometry and bacterial cultures from 3 replicates, obtaining $1.12x10^{10}$ live bacteria per capsule immediately after preparation and $1.44x10^{10}$ after 3 months kept at 4ºC.

The full documentation on the processing, preparation and viability control of the FMT product is provided elsewhere. The product is in patent proceedings.

7.4. Storage conditions

Vivomixx® must be stored between 2ºC and 8ºC protected from light.
FMT capsules are stored at 4ºC.

7.5. Concomitant therapies

Any concomitant therapy must be recorded in the CRF detailing product, dates, dosage, route of administration, indication.

Nonpermitted medication
Use of antibiotics other than the 7-day decolonization course of oral non-absorbable antibiotics (NAA) is forbidden during the study duration.

8. STUDY DEVELOPMENT AND ASSESSMENTS

8.1. Study endpoints

Primary endpoint

Proportion of patients with digestive decolonization rate defined as negative rectal swab (RS) for the target MDR-GNB (ESBL-producing Klebsiella pneumoniae, CPE and MDR/XDR Pseudomonas aeruginosa) at the end of study (60 ± 7 days after the randomization).

Secondary endpoints

1.- Proportion of patients with digestive decolonization rate defined as a negative fecal sample for the target MDR-GNB at the end of study treatment (3 weeks after randomization).
2.- Proportion of patients with digestive decolonization rate defined as a negative rectal swab for the target MDR-GNB after the decontamination period with non-absorbable antibiotics (week 1 after randomization).
3.- Proportion of patients with target MDR-GNB or any other MDR-GNB in any control RS or fecal sample during the study period.
4.- Proportion of patients with clinical infections during the study period.
5.- Proportion of patients with development of resistance to colistin or amikacin in any strain isolated in control RS or fecal sample during the study period.
6.- Changes from baseline in fecal microbiota composition at the end of study treatment.

8.2 Study development

Table 1 shows the summary of visits and procedures that will take place during the study.

1. Inclusion visit (from day -7 to day -1). Patients admitted to the Hospital Clínic with a positive rectal colonization within the previous 7 days by rectal swabbing will be assessed for eligibility. Eligible patients will be invited to participate into the study. If they accept, informed consent will be obtained. After providing informed consent, the following data will be collected: demographic data (age and sex), medical history, concomitant medications and comorbidities and a pregnancy test in urine will be carried out in childbearing women.

2. Baseline visit (day 0). Eligible patients will be randomized to one of the three study arms.
During the visit, a fecal sample will be obtained for MDR-GNB detection and the analysis of microbiota. Patients will be instructed to initiate the treatment with NAA during 7 days.

3. Treatment initiation visit. After the decontamination period, a treatment initiation visit will be conducted. The visit will be carried out 1-3 days after the last dose of NAA. During the visit, patients assigned to the probiotic arm will start the probiotic treatment and those patients assigned to the FMT arm will start the first dose of the FMT preparation.
Patients assigned to the Probiotic arm will receive 2 sachets of Vivomixx® every 12 hours (orally or through a nasogastric tube) during 14 consecutive days.
Patients assigned to the FMT arm will receive the first dose of the FMT preparation (orally or through a nasogastric tube) consisting of 14-17 capsules.
Patients assigned to the observation arm will neither receive probiotic nor FMT.
Prior to the initiation of the treatment study, a rectal swab will be carried out.
During this visit adverse events will be assessed.

4. Treatment follow-up visit (from day 15 to day 16). Those patients assigned to FMT arm will start the second dose of the FMT preparation (orally or through a nasogastric tube) consisting of 14-17 capsules. A second rectal swab will be carried out prior to the initiation of the second dose of the FMT preparation.

5. End of treatment visit. A follow-up visit will be scheduled once the 2-week treatment period is completed (22 to 23 days with a window of 7 days after randomization). During the visit a fecal sample will be obtained for MDR-GNB detection and analysis of microbiota and adverse events will be assessed.

6. End of study visit (60 days after randomization). The visit will be carried out 60 days (± 7 days) after randomization. A rectal swab will be carried out and adverse events will be assessed.

Table 1. Visit schedule

VISIT	Inclusion (Visit 0)	Baseline (Visit 1)	Treatment initiation visit (Visit 2)	Treatment follow-up visit* (Visit 3)	End of treatment visit (Visit 4)	End of study visit (Visit 5)
Day	-7 to -1	0	8 (window visit +3 days)	15 to 16	22 to 23 (window visit +7 days)	60 (window visit ±7 days)
Inclusion criteria	√					
Informed consent	√					
Physical examination	√		√		√	√
Medical history	√		√		√	√
Randomization	√	√				
Rectal swab	√		√	√		√
Fecal sample		√			√	
Treatment with NAA		√				
Probiotic/FMT administration			√	√		
Adverse events		√	√		√	√

* only in patients assigned to TMF arm

8.3 Procedures and follow-up

Patients will be followed on a daily basis during admission, once per week after discharge until the end of treatment and once per month until the end of the study. After patient discharge, scheduled visits will be done at a specialized day-care clinic.

Administration of FMT will always be performed under direct observation of one of the investigators and if ambulatory, the patient will remain under observation in the clinic during at least 2 h after the procedure. Accordingly to the standard FMT procedure in our Centre, on the evening before the the administration of the study FMT product and on the morning of the day of FMT product ingestion, the participant will take 20 mg of omeprazole by the oral or iv route. On the morning of the day of FMT, the patients will also receive a commercial laxative enema of 250 mL.

Ambulatory patients will be instructed on the preparation of the probiotic at home to be taken without direct observation. The empty sachets will be spared and counted at the corresponding scheduled visit.

In all patients included in the study and throughout the study period, a prospective registry of adverse events will be performed, including the presence of diarrhea and *C. difficile* infection

8.3.1. Clinical variables

From all included patients, the following variables will be collected: demographics (age and sex), comorbidities, date of admission to the hospital and ICU, reason for admission to the hospital and ICU, previous admissions in the last 6 months, antibiotics received in the previous month, any invasive procedure performed during the present admission (intubation and

mechanical ventilation, surgery, central venous catheterization, bladder catheterization, other), infections acquired during the study period and antibiotic treatments administered during the entire study period.

8.3.2. Microbiological studies

Rectal swabs will be seeded on selective chromogenic culture and non-selective plates to detect ESBL-producing, carbapenemase-producing, cefamicinase or chromosomal beta-lactamase-producing Enterobacterials and *Pseudomonas aeruginosa*. The identification of microorganisms at gender and species level will be carried out using the MALDI-ToF system (Bruker Daltonics). Confirmation of antibiotic resistance will be done by phenotypic methods including the disc-plate, E-test and/or marketed panels of the Phoenix system (Becton Dickinson) or Sensititre (Thermo-Fisher). Molecular epidemiology studies by using pulsed field electrophoresis and MLST analysis will be performed to establish the clonality of clinical and rectal isolates. The detection of the mecanism of resistance to betalactams will be investigated by PCR genotypic methods. After the detection of beta-lactamases, specific PCRs will be carried out to be able to sequence the detected enzyme. In addition, a group of multiresistant strains will be studied through massive sequencing to know the various genes involved.

8.3.3. Control rectal swabs (RS)

In all study arms, a RS will be obtained at the end of the NAA regimen (treatment initiation visitday 8 after randomization) prior to FMT or starting the probiotic and at the end of the study (60 ± 7 days after randomization). In patients assigned to the FMT arm, a RS prior to the second FMT (pre-2FMT) will also be performed to have a better dynamic profile of carriage after the first FMT and to confirm if there is a rebound of colonization at that point. We will consider the patient as decolonized when the RS is negative at the end of the study visit (60±7 days after randomization). In all positive control rectal swabs or rectal sample, the presence of target MDR-GNB will be determined, as well as their susceptibility to colistin and amikacin.

8.3.4. Analysis of the microbiota

A microbiota analysis will be carried out in all the patients from the intervention groups to evaluate the degree of fecal microbiota restoration, engrafment success and relationship of fecal composition with MDR-GNB eradication from baseline at the end of treatment (at day 22-23 with a window of 7 days after randomization).
Fecal samples at baseline and at the end of study treatment will be obtained and stored to conduct the analysis of the microbiota.

8.4. Samples for analysis at the end of the study

Fecal samples for analysis of microbiota will be obtained at baseline and the end of treatment visit.
Faecal samples will be frozen within the first 6h of the collection at -80 ° C. The PureLink ™ microbiome DNA purification kit (Invitrogen) will be used for the DNA extraction according to the manufacturer's instructions. The DNA concentration will be normalized for all samples after quantification with a Quantus™ Fluorometer (Promega) and the QuantiFluor® ONE DNA System (Promega). KAPA HiFi HotStart Ready Mix (Kapa Biosystems) will be used to amplify the V3-V4 region of 16S rRNA. The PCR products will be sequenced using the 300 bp paired-end technique with the Nextera XT kit (Illumina) following the standard protocol provided by Illumina and sequenced on a MiSeq platform (Illumina) using a Miseq v3 reagent kit (Illumina). The quality of the sequencing data will be evaluated with the fastqc and prinseq-lite programs. R1 and R2 will be joined using flash. The taxonomic affiliations will be assigned using the rdp_classifier

and the RDP database. At the same time, we will calculate the relative abundances of the operational taxonomic units (OTU) with the QIIME pipeline. The phylogenetic classification will be used to describe the intestinal composition of each subject in the different groups. The microbial diversity will be calculated by calculating the Shannon diversity index (SDI). All calculations and statistics will be carried out within the R environment.
After analysis, the fecal samples will be destroyed in accordance with Law 14/2007 of 3 July on Biomedical Research.

9. SAFETY

9.1 Definitions

Despite the study is not assessing the effect of medicinal products, the unwanted effects will be recorded in accordance with the existing definitions for medicines.

Adverse event (AE): an AE is any untoward medical occurrence in a clinical study subject administered the investigational product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of the investigational product, whether or not related to that product
This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Serious adverse event (SAE): a SAE is any untoward medical occurrence that at any dose: Results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, is a suspected transmission of any infectious agent via a medicinal product, is medically important (according to the treating physician), other important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.
If a serious and unexpected AE occurs for which there is evidence suggesting a causal relationship between the study drug and the event (eg, death from anaphylaxis), the event must be reported as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (eg, all-cause mortality).
Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a study must be reported as an SAE, except hospitalizations for the following: hospitalizations not intended to treat an acute illness or AE (eg, social reasons such as pending placement in long-term care facility), surgery or procedure planned before entry into the study.

9.2 Assessment of intensity

An assessment of intensity grade will be made using the general categorical descriptors outlined in the WHO Toxicity Grading Scale (see Appendix 1). The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (eg, laboratory abnormalities).

9.3 Assessment of causality

- AE related: The relationship in time of the AE with the study treatment indicates a possible

causal relationship and it cannot be explained by factors such as the patient's clinical condition or therapeutic interventions.

- AE unrelated: The relationship in time of the AE with the study drug indicates an unlikely causal relationship, or other factors (concomitant medication or conditions) or other therapeutic interventions provide a satisfactory explanation for the AE.

9.4 Collection and follow up of adverse events

AEs may be recorded at each visit based on careful clinical observation of the patient, laboratory tests or spontaneously reports by the participant discovered as a result of general questioning by the study staff. All AEs will be recorded on the medical history and in the CRF. The investigator will also decide whether the adverse event is, based on his/her judgment, related or not to the study drug—this decision should also be noted in the medical history and CRF.
The following will be recorded for each event: description, severity (grade 1, 2, 3, 4 and 5), duration (start and end dates), intensity, causal relationship with the drug (according to the previously attributability criteria) and study drug(s) for which this causal relationship is suspected, actions taken and outcome, using choices given on the patient's medical history. The investigator should report the underlying condition when a surgical or medical procedure is required as the event term, and the procedure as an action taken. For a preexisting AE that has worsened in terms of severity or frequency, the meaning of the change should be specified.
For all AEs, the Investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets criteria for classification as a serious adverse event (SAE). Follow-up of the AE is required if the AE persists until the event resolves or stabilizes at a level acceptable to the Investigator.
The degree of severity of an adverse event provides a qualitative assessment of the extent or intensity of an adverse event elicited by the investigator or reported by the patient. Severity does not reflect the clinical seriousness of the event, only the grade or extent of the complaint or incidence.

9.5. Procedure for reporting serious adverse events

The principal investigator or delegates will report immediately any SAE occurring from signing of informed consent and up to 30 days after the end of the study to the CTU Clinic.
The initial report of SAE should be written and as complete as possible including details of the current disease and SAE and assessment of the causal relationship between the AE and the study procedure. Reporting will be made using the Serious Adverse Event Report Form included in Appendix 2 of this protocol within 48 hours from first knowledge by the investigator, completing all information on the form in the following two days.
The information missing at the time of the initial report must be reported in the SAE follow-up form.
SAEs forms should be sent immediately by fax or email to:

CTU CLINIC
Hospital Clinic de Barcelona
Fax number: +34 93 227 98 77
Email address: jpich@clinic.cat

10. DATA COLLECTION AND PROCESSING

10.1. Recording of data

All data required for the study will be recorded in the participating center using a case report form (CRF). Completeness and plausibility checks will ensure the collection of high quality data. A CRF for each patient will be completed by authorized personnel who must be identified and authorized in writing by the Principal Investigator before they conduct any study related tasks. A delegation of responsibility log identifying who can enter data and/or sign off a CRF will be maintained by the Principal Investigator.
The subject's number and date of entry into the study, along with a study identifier, should be recorded in the subject's study records. The following should also be recorded in the study records: confirmation of written consent, the subject's clinical status, date of every study visit, copies of all relevant reports, comments on results and reference to serious adverse events and related adverse events.

10.2. Direct access to source data/documents

Investigators will ensure access to the source documents of the staff responsible for guaranteeing data quality and data analysis. In addition, access to documentation will be provided, if necessary, to the staff duly authorized by the sponsor (study monitors).

10.3. Data management

The Investigator must ensure the accuracy, completeness, legibility and timelines of data reported in the CRF and all required reports.

10.4. Archiving and storage of data

The investigator is responsible for maintaining all records which enable the conduct of the study at the site to be fully documented, in accordance applicable national regulatory requirements. Timeliness and completeness of the documentation will be regularly checked by the clinical monitor. All completed study related documents (e.g. eCRF, Informed consent forms, Subject identification log, etc) must be archived at site.

11. QUALITY CONTROL

The purpose of monitoring is to verify that the rights and wellbeing of human subjects are protected; that the study is accurate, complete and verifiable with source data and that the study is conducted in compliance with the protocol, and the applicable regulatory requirements. A monitoring plan will be designed. The monitoring plan will establish the guideline for conducting all the monitoring activities.
Source data will be verified during on-site monitoring visits. During the visits, the monitor will compare the data entered into the CRF with the source documents. The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the monitor and study-site personnel and are accessible for verification. During monitoring visits, the relevant study-site personnel should be available, the source documentation accessible, and a suitable environment provided for review of study-related documents.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Ethics approval

This study will be conducted in accordance with the protocol and ethical principles stated in the Declaration of Helsinki and all applicable local laws, rules, and regulations.
The protocol, informed consent form, and other required documents will be approved by the Ethics Committee (Comité de Ética de la Investigación, CEI) of the Hospital Clínic de Barcelona before enrolment of subjects in the study.
Protocol amendments must not be implemented without prior Ethics Committee approval.

12.2. Reponsabilities of the investigators

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, and applicable regulatory Spanish requirements.
In addition, the investigator is responsible for giving information about the study to all staff members involved in the study or in any element of subject management, before and during the study.

12.3. Patient confidentiality

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study. Patient data will be anonymized to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

Subjects will be codified with a study code that prevents their identity from being deduced.

The PI and duly authorized collaborators will compromise to maintain personal data strictly confidential, according to the corresponding country-specific requirements. The link between the numeric code and real personal data from subjects will be rigorously kept by the PI. The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, Ethics Committee review, and regulatory inspection.

In the case report form, the patient will only be identified by the assigned study code. The name of patients will not appear in any publication or report of the study results.

The participation of the patient in the clinical research will be noted in their medical records.

The investigator will complete a list which will include the names of the patients participating in the trial, the number of inclusion in the study, and their medical history. Only investigators and the staff responsible for guaranteeing data quality and data analysis will have access to the clinical documentation of the participants.

Duly authorized persons by the sponsor and the health authorities and the Ethics Committee may audit or inspect the clinical research. Personal information will not be publicly available, in compliance with General Data Protection Regulation (GPDR) (EU) 2016/679 of 27th April 2016).

12.4. Informed consent

Informed consent will be obtained from all participating individuals or their representatives. Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The ICF(s) must be signed before performance of any study related activity. The ICF(s) that is/are used must be approved by both the sponsor and by the reviewing Ethics Committee and be in a language that the patient can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki (updated according to its last version, Fortaleza, Brazil, 2013), , applicable regulatory and country-specific requirements, and sponsor policy.

Before enrollment in the study, the investigator or an authorized member of the study-site personnel must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort that the participation in the study may entail.

Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his or her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that the investigator will maintain a subject identification register and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the subject.

12.5. Personal data protection

The processing, communication and transfer of personal data of all participants will be adjusted to compliance with Regulation EU 2016/679 of the European Parliament and the Council of 27 April 2016 on the protection of natural persons as to the processing of personal data and the free circulation of data, being mandatory as of May 25, 2018. The legal basis that justifies the processing of the patient data is the consent given in this act, in accordance with the provisions of the Article 9 of the EU Regulation 2016/679.
The data collected for the study will be collected only identified by a code, so that no information will be included to identify the participants. Data will be processed with the only purpose of carry out all activities related to the clinical research. The legal basis for the processing of the data is the participant consent and Article 9.2 of the Regulation. Only the study doctor and his collaborators have the right to access the source data (clinical history) and will be able to relate the data collected in the study with the patient's medical history.
The identity of the participants will not be available to any other person except for a medical emergency or legal requirement. Health authorities, the Ethics Committee and personnel authorized by the Sponsor of the study, may have access to the personal data identified when necessary to verify data and study procedures, but always maintaining confidentiality in accordance with current legislation.
Only encrypted data will be transferred to third parties and to other countries, which in no case will contain information that can identify the participant directly (such as name and surnames, initials, address, social security number, etc.). In the event that this assignment occurred, it would be for the same purpose of the study described and guaranteeing confidentiality. No transfer data will be carried out outside the EU.

The sponsor of the trial commits to carry out the data processing according to EU Regulation 2016/679 and, therefore, to keep a record of the processing activities to carry out and to make a risk assessment of the data processing, to establish what measures will be applied and how it will be done.
In addition to the rights already covered by the previous legislation (access, modification, opposition and cancellation of data, deletion in the new Regulation) participants can now limit the processing of data collected for the project that has to be rectified, request a copy or move to a third party (portability). To exercise these rights, the participant should be directed to the principal investigator of the study or the Data Protection Delegate of Hospital Clínic de Barcelona. The participant also has the right to contact the Data Protection Agency if not satisfied.
The data cannot be deleted even if a patient discontinues the study, to guarantee the validity of the investigation and comply with the legal duties and the medication authorization requirements.
The Investigator and the Sponsor are obliged to keep the data collected for the study at least up to 25 years after its completion. Subsequently, personal information will only be kept by the site for the care of their health and by the sponsor for other scientific research purposes if the patient has given their consent to do so, and if the law and applicable ethical requirements so permit.
In accordance with the provisions of recital 33 of the regulations and the corresponding provisions of each country involved in the study regulations, the data may be preserved in such a way that the clinical data are kept separate from the identifiers, to be used in future investigations, applying all technical precautions necessary to avoid their re-identification, and in accordance with all ethical and legal requirements.

13. FINANCING, INSURANCE AND PUBLICATION

13.1. Financing

This study is partially funded by a public grant (*ISCIII Acción Estratégica de Salud* PI19/00842).

13.2. Insurance

No specific insurance provision is required because an increased risk for patients beyond that associated with standard clinical practice is not expected.

13.3. Publication

The main results will be published by the sponsor in accordance with the Spanish requirements.

14. STATISTICS

14.1 Number of participants and sample size calculation

According to our clinical and rectal surveillance data and assuming a 75% rectal colonization rate in patients with positive clinical samples and a loss of 20% of putative participants, we

estimate to include 437 patients in total, 175 patients per treatment arm and 87 as a control group.

In the intervention arms, the expected distribution will be 25 CPE carriers per arm, 75 patients carrying ESBL-*Klebsiella pneumoniae* per arm and 75 patients carrying MDR/XDR *Pseudomonas aeruginosa* per arm. In the control arm, half these figures are expected. With this sample size and taking into account a decolonizing effectiveness of 30%-50% obtained in previous studies, the study would have a power of 80% to detect a difference between treatment arms of 15 percentage points and a difference between treatment and control arms of 20 percentage points at a significance level of 5%.

Diference 50% vs 30%: 69/129 per control/treatment arms
Diference 40% vs 20%: 59/118 per control/treatment arms
Diference 30% vs 10%: 44/88 per control/treatment arms

14.2 Statistical analysis

For the primary outcome the main analysis will be performed according to **the intention to treat** principle, considering as intention-to-treat population all randomized patients. Missing RS will be considered as positive. In addition, we intend to do a **per protocol** analysis, evaluating only the patients who end the treatment and end the study. Categorical variables will be compared by the Fisher exact test and continuous ones the the student t-test or Mann-Whitney test. Univariate logistic regression with group assignment as predictor variable and clinical characteristics and decolonization as outcome will be performed to calculate OR and 95% confidence intervals. In the event of imbalance in the distribution of potential confounder despite randomization, a multivariate logistic regression analysis with decolonization as de dependent variable and group assignment as one of the explanatory variables will be carried out.

All randomised patients will be included in the "intention-to-treat population" for analysis. Patients withdrawn for any reason or lost to follow-up will be considered as treatment failure in this analysis.

Subgroup analyses

An analysis of efficay for each bacterial species/resistance determinant (ESBL-producing *K. pneumoniae*, carbapenemase-producing *Enterobateriaceae*, MDR-*P. aeruginosa*) are planned

Interim analysis

A safety interim analysis is planned after inclusion of the first 100 individuals in the study.

Analysis Centre

Data will be analyzed in the Institut Clínic de Medicina Interna, Dermatologia I Infeccions del Hospital Clínic de Barcelona.

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