## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | 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). |
| **AUTHORS** | Pérez-Nadales, Elena; Cano, Ángela; Recio, Manuel; Artacho, María José; Guzmán-Puche, Julia; Doblas, Antonio; Vidal, Elisa; Natera, Clara; Martínez-Martínez, Luis; Torre-Cisneros, Julian; Castón, Juan José |

## VERSION 1 – REVIEW

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| **REVIEWER** | Palomba, Emanuele<br>IRCCS Foundation Maggiore Policlinico Hospital, Infectious Diseases |
| **REVIEW RETURNED** | 27-Nov-2021 |

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| **GENERAL COMMENTS** | The study protocol by Perez-Nadales et al. addresses the current issue of MDRO decolonization strategies. FMT has been considered as a tool to achieve decolonization in several case series and case report but to date there is still lack of adequate evidence coming from RCTs.<br>Overall, the study protocol is well designed, even though the sample size might not be sufficient to achieve a sufficient study power (please expand on the previous unpublished results of the KLEBCOM study, line 334, regarding the rate of spontaneous decolonization).<br>The major weakness of the study is not addressing the issue of donor selection for FMT material (a well-known problem since the publication of the work by DeFilipp et al. in 2019). Will the donor be screened with microbiological/molecular enquires? How the donor selection process will be organised? Please expand on this matter.<br>Furthermore, the choice of excluding immunocompromised patients and HSCT recipients from the study protocol will prevent from getting information about key populations, particularly affected by the MDRO threat (as stated in the protocol introduction, line 95). Please justify this decision and add it to the limitations paragraph.<br><br>Minor suggestions:<br>1- The authors should consider adding as secondary outcome a subgroup analysis on how subsequent antibiotic administration impact on the colonization status.<br>2- Lines 104-106, add reference |

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| | 3- Line 109, Clostridioides difficile is misspelled; moreover, the germ is not a MDRO<br>4- Lines 121-123, the definition of FMT can be improved<br>5- Line 272, FMT is misspelled<br>6- Line 297, enrollment is misspelled<br>7- Line 389, discussion can be improved expanding on the use of FMT for decolonization in the immunocompromised host (i.e. Alagna et al. doi: 10.3390/ijms21165619)<br>8- Line 418, Clostridioides difficile is misspelled<br><br>Finally, the protocol would benefit from a thorough language revision. |

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| **REVIEWER** | zhang, faming<br>the Second Affiliated Hospital of Nanjing Medical University, Medical Center for Digestive Diseases |
| **REVIEW RETURNED** | 04-Dec-2021 |

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| **GENERAL COMMENTS** | The phase II, double-blind, placebo-controlled clinical trial was designed to assess the efficacy and safety of oral FMT capsules to eradicate the selective intestinal colonization of KPC carbapenemase-producing Klebsiella pneumoniae (KPC-Kp). It is interesting and valuable because the rectal colonization with CRE is an important risk factor for the development of subsequent CRE infection, and FMT is an antibiotic-free decolonization strategy which has been demonstrated to be effective for MDRO. Here are my questions regarding this study.<br>1. Although the authors explain the rationale for the dose of FMT capsules used in the discussion, based on experience and reports from other studies, the lyophilized capsules used in this study were not sufficient. Was the same dose used in previous studies at the author's institution? If the efficacy of this dose was supported by previous studies, please clarify in this article.<br>2. In introduction, the FMT delivery routes did not cover another important novel delivering way---colonic transendoscpic enteral tubing.<br>3. Inclusion criteria: Absence of KPC-Kp clinical samples at the time of informed consent and in the previous month. What is the purpose of this inclusion criterion?<br>4. Exclusion criteria: The exclusion criteria in lines 233 and 242 can be combined to make it more concise.<br>5. Primary outcome should specific to the rate of KPC-Kp eradication at 30 days.<br>6. There are some minor errors need to clarify:<br>(1) Headings should be concise and stress the key point. What does "KAPEDIS study" refer to? Please revise the title.<br>(2) Abbreviations should be marked with full name when first appeared, such as NDM, MIC, and others. Some abbreviations that have been marked with their full name are not necessary to be explained again, such as CPE, ESBL-E, MDRO, FMT, and etc. Please check them carefully.<br>(3) Line 113-114 and 121-123 require corresponding references. In line 135 and 247, correct the CPE to CRE. Please check full manuscript and make sure the right spelling.<br>(4) Line 272 “FMT” not “TMF”<br>(5) Line 413 References should be cited in a consistent format that meets the requirements of the BMJ open.<br>(6) In the discussion section, the author used "CPE" instead of "KPC-Kp" many times. Is it a clerical error or other reasons? The two words are related but not identical. |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1
Dr. Emanuele Palomba, IRCCS Foundation Maggiore Policlinico Hospital Comments to the Author: The study protocol by Perez-Nadales et al. addresses the current issue of MDRO decolonization strategies. FMT has been considered as a tool to achieve decolonization in several case series and case report but to date there is still lack of adequate evidence coming from RCTs. Overall, the study protocol is well designed, even though the sample size might not be sufficient to achieve a sufficient study power (please expand on the previous unpublished results of the KLEBCOM study, line 334, regarding the rate of spontaneous decolonization).
The major weakness of the study is not addressing the issue of donor selection for FMT material (a well-known problem since the publication of the work by DeFilipp et al. in 2019). Will the donor be screened with microbiological/molecular enquires? How the donor selection process will be organised? Please expand on this matter.

Response: First of all, we would like to sincerely thank Dr. Emanuele Polomba for the revision of our paper.

Sample size calculation: Thank you for this comment. As stated in our protocol, for sample size calculation we assumed a "decolonization rate at 30 days of 30% in the control group and 60% in the experimental group". This assumption was based on two previous studies:

- Our previous KLEBCOM cohort study: Results from the KLEBCOM cohort have recently been submitted for publication. In this prospective observational cohort we recruited 80 elderly patients with intestinal colonization by KPC-KP and we observed spontaneous decolonization rates of 12%, 36% and 65% at one, three and six months of follow-up, respectively.
- A recent metanalysis conducted by Bar-Yoseph and collaborators showed CRE colonization rates of 73.9% (95% CI 64%-81.8%) at 1 month, 74.6% (95% CI 56.6%–86.9%) at 3 months and 55.2% (95% CI 37.3%–71.9%) at 6 months of follow-up, in observational studies without any intervention.

Based on these findings, for sample size calculations, we considered a 30% spontaneous decolonization rate in the control group. Since our KLEBCOM is yet not published we have eliminated this reference and have instead included a reference to the Bar-Yoseph *et al* paper.

The issue of donor selection for FMT: We agree with the reviewer that this information needs further clarification. We have incorporated a full new section in the main text entitled: "Donor selection". In addition, specific questionnaires employed for donor selection have been included in Supplemental Material:

- Supplementary Table S1. Donor Questionnaire
- Supplementary table S2. Interview with the donor
- Supplementary table S3. Microbiological screening for donors

Dr. Emanuele Palomba: "Furthermore, the choice of excluding immunocompromised patients and HSCT recipients from the study protocol will prevent from getting information about key populations, particularly affected by the MDRO threat (as stated in the protocol introduction, line 95). Please justify this decision and add it to the limitations paragraph".

Response:

- At the time of submitting this protocol to our IRB review Board, we could not justify including HSCT patients for two reasons: (1) the evidence of the safety of FMT in this patient population was scarce; (2) it would be hard to recruit enough HSCT patients in our hospital to obtain statistically relevant results of sound relevance for this patient population that could justify their inclusion. Therefore, this possibility was disregarded. Given the current incidence of CRE colonization among HSCT patients, we estimate that a multicentric, multinational study would be necessary with a specific focus on this population.
- We have added a sentence at the end of the Discussion including this limitation: "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 (Alagna et al. doi: 10.3390/ijms21165619), given the single-center nature of this RCT, they would be insufficiently represented to obtain statistically significant results that could justify their inclusion."

Minor suggestions:

1- The authors should consider adding as secondary outcome a subgroup analysis on how subsequent antibiotic administration impact on the colonization status.

Response: This information is being recorded for each recruited patient as part of clinical follow-up and will certainly be analysed as a potential confounding factor in the planned analyses.

2- Lines 104-106, add reference

Response: It has been added. Thank you.

3- Line 109, *Clostridioides difficile* is misspelled; moreover, the germ is not a MDRO

Response: We have rephrased this sentence, thank you.

4- Lines 121-123, the definition of FMT can be improved

Response:

- Before: 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 [2–9].

- Now: Fecal microbiota transplantation (FMT) is an emerging therapy for targeting and modulating the human intestinal microbiota [10]. 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 [11]. 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 [2–9].

5- Line 272, FMT is misspelled

Response: Corrected.

6- Line 297, enrollment is misspelled

Response: Corrected.

7- Line 389, discussion can be improved expanding on the use of FMT for decolonization in the immunocompromised host (i.e. Alagna et al. doi: 10.3390/ijms21165619)
Response: This has now been done. As indicated before, we have added a sentence at the end of the Discussion including this limitation: “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 (Alagna *et al*. doi: 10.3390/ijms21165619), given the single-center nature of this RCT, they would be insufficiently represented to obtain statistically significant results that could justify their inclusion.”

8- Line 418, *Clostridioides difficile* is misspelled
Response: Corrected.

Finally, the protocol would benefit from a thorough language revision.

Response: We have performed a careful language revision.

Reviewer: 2
Prof. Faming Zhang, the Second Affiliated Hospital of Nanjing Medical University Comments to the Author:
The phase II, double-blind, placebo-controlled clinical trial was designed to assess the efficacy and safety of oral FMT capsules to eradicate the selective intestinal colonization of KPC carbapenemase-producing Klebsiella pneumoniae (KPC-Kp). It is interesting and valuable because the rectal colonization with CRE is an important risk factor for the development of subsequent CRE infection, and FMT is an antibiotic-free decolonization strategy which has been demonstrated to be effective for MDRO. Here are my questions regarding this study.

1. Although the authors explain the rationale for the dose of FMT capsules used in the discussion, based on experience and reports from other studies, the lyophilized capsules used in this study were not sufficient. Was the same dose used in previous studies at the author's institution? If the efficacy of this dose was supported by previous studies, please clarify in this article.

Response: First of all, we would like to sincerely thank Dr. Faming Zhang for the revision of our paper. Regarding the reviewer´s question, it is the first time that an FMT protocol is approved by our IRB board to be used in an RCT in our hospital. The efficacy of the selected dose, i.e. a single dose of 4-5 capsules containing the minimum dose of $2.1 \times 10^{11}$, is based on previous studies, i.e. Journal of Hospital Infection 105 (2020) DOI: 10.1016/jjhin.2019.12.022. We have included this reference in the Methods section.

2. In introduction, the FMT delivery routes did not cover another important novel delivering way---colonic transendoscopic enteral tubing.

Response: We read with interest the publication on this new delivery method and we have included this information in a new sentence in the Discussion: “Methods for FMT delivery include colonoscopy, nasoduodenal tub, colonic transendoscopic enteral tubing or oral capsules [13,38,39]”.

3. Inclusion criteria: Absence of KPC-Kp clinical samples at the time of informed consent and in the previous month. What is the purpose of this inclusion criterion?

Response: In this RCT we only include patients with intestinal colonization with KPC-Kp but no clinical signs of active infection.

4. Exclusion criteria: The exclusion criteria in lines 233 and 242 can be combined to make it more concise.

Response: Corrected, thank you.

5. Primary outcome should specific to the rate of KPC-Kp eradication at 30 days.

Response: Corrected.

6. There are some minor errors need to clarify:
(1) Headings should be concise and stress the key point. What does "KAPEDIS study" refer to? Please revise the title.

Response: We have amended the title:

Before: Efficacy and safety of fecal microbiota transplantation against placebo for Selective Intestinal Decolonization of patients colonized by KPC-producing *Klebsiella pneumoniae* (KAPEDIS study): Study protocol for a randomized, double-blind, placebo-controlled, phase 2, superiority clinical trial.

Now: 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)

(2) Abbreviations should be marked with full name when first appeared, such as NDM, MIC, and others. Some abbreviations that have been marked with their full name are not necessary to be explained again, such as CPE, ESBL-E, MDRO, FMT, and etc. Please check them carefully.
Response: Corrected

(3) Line 113-114 and 121-123 require corresponding references. In line 135 and 247, correct the CPE to CRE. Please check full manuscript and make sure the right spelling.
Response: Corrected

(4) Line 272 “FMT” not “TMF”
Response: Corrected

(5) Line 413 References should be cited in a consistent format that meets the requirements of the BMJ open.
Response: Corrected

(6) In the discussion section, the author used "CPE" instead of "KPC-Kp" many times. Is it a clerical error or other reasons? The two words are related but not identical.
Response: For consistency, we now use CRE all throughout the document

Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: I declared no conflict of interest.

## VERSION 2 – REVIEW

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| **REVIEWER** | Palomba, Emanuele<br>IRCCS Foundation Maggiore Policlinico Hospital, Infectious Diseases |
| **REVIEW RETURNED** | 12-Mar-2022 |

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| **GENERAL COMMENTS** | The revised version of the protocol clearly addresses previous suggestions.<br>No further revision is needed. |

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| **REVIEWER** | zhang, faming<br>the Second Affiliated Hospital of Nanjing Medical University, Medical Center for Digestive Diseases |
| **REVIEW RETURNED** | 26-Feb-2022 |

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| **GENERAL COMMENTS** | Nice revision. |