Iterative fecal microbiota transplantations for eradicating digestive colonization with carbapenemase-producing Enterobacteriaceae: is it worth it?

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Abstract:	Carbapenemase-producing Enterobacteriaceae (CPE) have emerged as a major source of bacterial resistance and their dissemination is a serious public health threat.1 It has also been demonstrated that those bacteria could disseminate outside the hospital setting. A large study including 34 hospitals in Spain demonstrated that a significant proportion of patients identified as colonized or infected with CPE during hospitalization probably acquired this multi-resistant organism in a nursing home during the period preceding their hospital admission.2 In addition, a recent review of the literature demonstrated that according to US-based studies, the percentage of CPE isolates that could be associated with the community ranged from 5.6 to 10.8%.3
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Iterative fecal microbiota transplantations for eradicating digestive colonization with carbapenemase-producing Enterobacteriaceae: is it worth it?

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To the Editor - Carbapenemase-producing Enterobacteriaceae (CPE) have emerged as a major source of bacterial resistance and their dissemination is a serious public health threat.[1] It has also been demonstrated that those bacteria could disseminate outside the hospital setting. A large study including 34 hospitals in Spain demonstrated that a significant proportion of patients identified as colonized or infected with CPE during hospitalization probably acquired this organism in a nursing home during the period preceding their hospital admission.[2] In addition, a recent review of the literature demonstrated that according to US-based studies, the percentage of CPE isolates that could be associated with the community ranged from 5.6 to 10.8%.[3]

We have recently demonstrated the less effective effect of fecal microbiota transplantation (FMT) on CPE as compared to vancomycin-resistant Enterococci (VRE) fecal carriage.[4] Those results are consistent with another recent study [5] conducted in six patients colonized with CPE and showing an eradication of the colonization in only 2/6 patients. In these studies, the decolonization procedure included only one FMT. One hypothesis is that a protocol including iterative FMT separated each other by a several-day latency could increase the effectiveness of the procedure.

Our objective was to evaluate the impact of iterative MFT for the clearance of CPE carriage in our mouse model of digestive colonization.

Ethical approval was obtained from the Ethical Committee in Animal Experimentation of Pays-de-la-Loire, France (reference: 2015041415088410/APAFIS 513) and conducted according to European directives concerning the use of animals in research (86/609/EEC).

Twenty-eight 8-week old mice (Swiss type) were used for the model. The normal digestive flora was disrupted with the daily oral administration of a combination of antimicrobial agents including vancomycin (50 mg/kg), metronidazole (25 mg.kg) and ceftriaxone (25 mg.kg) during 5 days (from day 1 to day 5). Mice were then randomized to receive a high inoculum (5.10^9 bacteria) of a strain of *Escherichia coli* producing a New Delhi metallo-β-lactamase -1 (NDM-1). Those bacteria were inoculated to the mice by oral gavage on day 4, day 5, and day 8. Mice were housed in individual cages.

FMT was collected daily from related (Swiss mice of the same age) untreated mice. Stool suspensions for FMT were prepared and stored as previously described.[4] On day 10, mice were randomized to receive FMT (14 mice) or placebo (14 mice). During the experiment, 4 series of FMT or placebo administration were administered to the mice. In each series, the mice received FMT or placebo once daily for three successive days (from day 10 to day 12, from day 23 to day 25, from day 37 to day 39, and from day 49 to day 51) by oral gavage with 200 µL of the stool suspension or 200 µL of saline.

Stools were collected 3 times a week until day 57 and were weighted for quantitative cultures. They were seeded on agar media (ChromID CARBA, bioMérieux, France) after serial dilutions for CPE screening. Bacterial identification of CPE colonies was controlled by using the MALDI-TOF technology (Vitek MS, bioMérieux). A mouse was considered decolonized when 3 successive stool samples (corresponding to 4 or 5 days of follow-up) were found negative for CPE.

The evolution of the percentage of colonized mice during the follow-up period was studied by the Kaplan-Meier analysis (SPSS software v15.0). The comparison

between the FMT group and the placebo group was performed by using the log-rank test. The comparison of the percentage of colonized mice in each group at day 57 was performed with the Fisher exact test. A *p* value < 0.05 was considered statistically significant.

The results are presented on figure 1. Overall, by considering the placebo effect corresponding to the natural clearance of CPE digestive colonization of mice, the iterative FMT series demonstrated a moderate impact on the decolonization kinetics (p = 0.22). However, by considering the difference between the FMT protocol and the placebo at the end of the follow-up period, the decolonization rates were more clearly different (50% (7/14) and 21% (3/14) respectively), although this difference was not significant (p = 0.11).

Due to the low number of mice included, this study only provides preliminary results. Moreover, the uncertainty of reproduction in humans of what is demonstrated in animals must be considered.

The iterative FMT allowed the eradication of CPE in 50% of colonized mice, which can be considered as a moderately convincing result. In a recent study [6] conducted with patients presenting blood disorders, the digestive carriage of NDM-1-producing *Klebsiella pneumonia* was eradicated in less than 50% (6/14) of cases. In our experiment, it is noteworthy that the decrease of the percentage of colonized mice was higher in the FMT group than in the placebo group for each of the 4 treatment series (figure 1). However, the short length of stay of most hospitalized patients limits the applicability of a decolonization protocol including iterative FMT in the clinical practice. However, it could be conceivable for patients hospitalized in long-term care

facilities or for the residents in nursing homes, in contexts of uncontrolled cross-transmission during certain outbreaks of CPE carriage, and if the FMT are administered orally. Additional studies are needed for evaluating the impact of the composition of the transplanted fecal material on the FMT outcome in terms of CPE eradication. Indeed, Ubeda et al.[7] have recently demonstrated that intestinal microbiota transplant containing Barnesiella species cured VRE colonization in mice.

Conflict of interest statement

No author declares conflict of interest.

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Figure 1: Comparative effect of iterative fecal microbiota transplantation and placebo administration on the digestive colonization with a carbapenemase-producing *Escherichia coli* in a mouse model.