# Review of: "Long-term beneficial effect of faecal microbiota transplantation on colonisation of multidrug-resistant bacteria and resistome abundance in patients with recurrent Clostridioides difficile infection"

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Potential competing interests: No potential competing interests to declare.

REVIEW:

Long-term beneficial effect of faecal microbiota transplantation on colonization of multidrug-resistant bacteria and resistome abundance in patients with recurrent Clostridioides difficile infection

**1. Summary**

The threat posed by multidrug-resistant (MDR) microorganisms is increasing. Faecal microbiota transplantation (FMT) is a beneficial method for treating various microbiota-related disorders as well as preventing the recurrence of Clostridioides difficile infections (rCDI). By combining culture techniques and faecal metagenomics, the investigators studied the impact of FMT in patients with rCDI on colonization with MDR bacteria and antibiotic resistance genes (ARG). Based on MDR culture, there was a reduction in the colonization rate of MDR bacteria after FMT. While there was a decrease in the relative frequency of ARGs in feces, the number of various resistance genes in patients was still larger than in healthy donors. Additionally, plasmid predictions in metagenomic data show that rCDI patients had higher concentrations of resistance plasmids, which didn't seem to be impacted by FMT. The resistomes of the recipients changed throughout time, demonstrating that microbiota restoration continued three weeks after FMT. The authors proposed that FMT eliminates possible pathogens or drives them to extremely low abundances while restoring the gut microbiota to a composition close to that of healthy donors. However, this process does not finish in the days after FMT. The gut microbiota may not achieve homeostasis for several months. FMT could result in a more consistent and robust microbiota composition, even while a pool of resistance genes still exists.

**2. General comments**

Overall, the results are very interesting, and the manuscript is written well. However, there are some minor comments to be made before publication. The manuscript is very well written and very well designed. The methodologies already reported by other authors are well described. With minor amendments and moderate adjustments to expand the results section, I recommend the study for publication. Limitations of this study: The generalibility of the conclusion may be limited, the authors should add new and actual references. The authors also need to add the ethical statement.

**3. Constructive criticism**

Abstract

- It would be interesting to add the overall practical implementation of your observation at the end of the abstract.
- Does it need keywords?

Introduction:

- Please indicate the main aim of the study.
- The review is an aggregation of selected information from publications which may be potentially useful to newcomers to the field; however, there appears to be little contemporary citations as would be appropriate for an introduction.

Results

- Each result in the experiments is presented in a good way.
- Figures are very good.

Discussion:

- I would separate the discussion in sub-sections to allow easier reading.
- It would be desirable if the authors could give an outlook regarding the relation between multidrug resistance bacteria and *Clostridioides difficile.*
- The discussion should be more comprehensive.
- I think the conclusion must provide us with the applied implication of your results in a concise manner.
- Please add at the end of the manuscript the limitations of your work: what can't the results and discussion tell us?