# 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.

1. The paper of Nooji et al. describes the beneficial effect of FMT against mainly C. difficile infection patients who harbours a plenty of MDR bacteria and antibiotic resistance genes inside their gut. Their study is only limited to beta-lactamase-producing (ESBL) Enterobacterales group of bacteria. The global resistance scenario of the gut is not described in this article, which I think a potential drawback of this manuscript. Because in the title they are claiming “Long-term beneficial effect of faecal microbiota transplantation on colonisation of multidrug-resistant bacteria and resistome abundance in patients with recurrent Clostridioides difficile infection”, in this scenario they should be more specific while writing the title, on the other way authors should work on the other MDR bacteria and resistome profile.
2. Authors mentioned antibiotic treatment cause C. difficile infection, when the gut microbiota is perturbed due to antibiotic treatment. But the major question I have, is there any relation between C. difficile infection and increase in the no. of MDR genes in bacteria? Is somehow C.difficile infection increasing the no. of more MDR genes and bacteria inside the gut? If possible, Authors should add a group of patients who do not have C.difficile infection but treated with antibiotics for long term to answer the above question.
3. How the authors determined the sample size and the dose of FMT that is not clear from the methodology? How many times the FMT was given to a single patients authors should mention that too.
4. Do you think the sample size is good enough for the claim what you claimed? Because there is no decrease in the resistance genes, rather increase in the resistance plasmid in the FMT treated group with time. So is really FMT has a long term or permanent beneficial effect as a controlling measure of MDR genes among gut bacteria?
5. How the authors choose the healthy individuals as the donor for FMT? What are the parameters or inclusion-exclusion criteria they have kept in mind at the time of choosing donor?
6. Before FMT author treated the patients with different types of antibiotics. Why they have given different antibiotics to different patients? Why not a single type of antibiotic for all the patients? Do you not think the 4 days antibiotic treatment is good enough to cause more severe C. difficile infection, and more no. of MDR genes and bacteria inside gut?
7. Is your data paired? If not, then why you have done paired t-test?
8. FMT is not actually neither reduce the MDR bacteria, whereas it is actually increasing the no. of resistance plasmid and ultimately producing more persister cells, which is an even more dangerous threat for future. What is the authors

take on it?

9. In fig. 2B the LFTU data is very and thus non conclusive. I also have the doubt on the significance level calculation when comparing Pre and Post FMT with LFTU group.
10. In Fig. 3A, in the PCA plot, there is no changes in the overall gut microbial composition while comparing the Post-FMT and LFTU group. What is your take on it? Because it is really very strange for me that after 2 years of FMT, the total microbial abundance is similar with the Post-FMT group.