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.

The manuscript “Long-term beneficial effect of faecal microbiota transplantation on colonisation of multidrug-resistant bacteria and resistome abundance in patients with recurrent Clostridioides difficile infection” by Nooij et al. describes the effect of FMT on reducing the abundance of antibiotic resistance genes in the short term, while facilitating a long-term transition to a donor-like gut microbiota. The effect of FMTs on reducing the harmful effects of recurrent C. difficile infections has shown promise in past studies. The current study expands on previous work to look at long term effects of FMT's, particularly as it relates to the proliferation of antibiotic resistance genes. The manuscript can be improved with the following considerations:

The introduction is focused on the use of antibiotics increasing colonization by C. difficile and in the proliferation of antibiotic resistance genes. It may be worth to have a paragraph about antibiotic associated increases in other pathogens, such as those that cause urinary tract infections, and antibiotic associations with chronic disease. C. difficile infection and antibiotic resistance are only 2 pieces of the puzzle.

For the culturing of MDR bacteria from stool, it is unclear how scraping stool with an inoculating loop would consistently yield 10uL of stool. Was this process validated and standardized? If so, then how?

For whole genome sequencing, only 24 out of a total of 30 isolates were used for sequencing. This seems like a very low number considering that there were MDR cultures were generated from 87 patients. Was whole genome sequencing just used to qualitatively characterize some of the resistant bacteria? Are the chosen bacteria representative of what was typically cultured from patients? Author's use these whole genomes for quantitative metrics using shotgun metagenomic data, so information on how typical the isolates are in the patients would be valuable information.

“Enterobacterales prevalence dropped in the weeks after FMT (58/63 = 92%; $p < 0.0001$; figure 3A)” - I think this should be figure 2b

Authors used viralVerify to predict plasmid-mediated resistance genes. As the name implies, this software classifies contigs as viral or non-viral. It also has a function to classify plasmids. However, this is non-obvious given the name. It might be worth to explain that algorithm a bit more and why it was used.