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Intervertebral disc microbiome in Modic Changes: lack of result replication underscores the need for a consensus in low-biomass microbiome analysis

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1. Background

The emerging field of the disc microbiome challenges traditional views of disc sterility, which opens new avenues for novel clinical insights. However, the lack of methodological consensus in disc microbiome studies introduces discrepancies.

2. Aims:

- To compare the disc microbiome of non-Modic (nonMC), Modic type 1 change (MC1), and MC2 discs to findings from prior disc microbiome studies
- To investigate if discrepancies to prior studies can be explained with bioinformatic variations

3. Methods

Seventy discs (24 nonMC, 25 MC1, and 21 MC2) were collected from spinal fusion surgery patients. The experimental setup included buffer contamination controls and was performed under aseptic conditions. The individual steps are listed in Figure 1. Our methodology and results were contrasted with previous disc microbiome studies (Rajasekaran et al. (2023)). Critical bioinformatic steps that were different in our best-practice approach and previous disc microbiome studies (taxonomic lineage assignment, prevalence cut-off) were varied and their effect on results were compared.

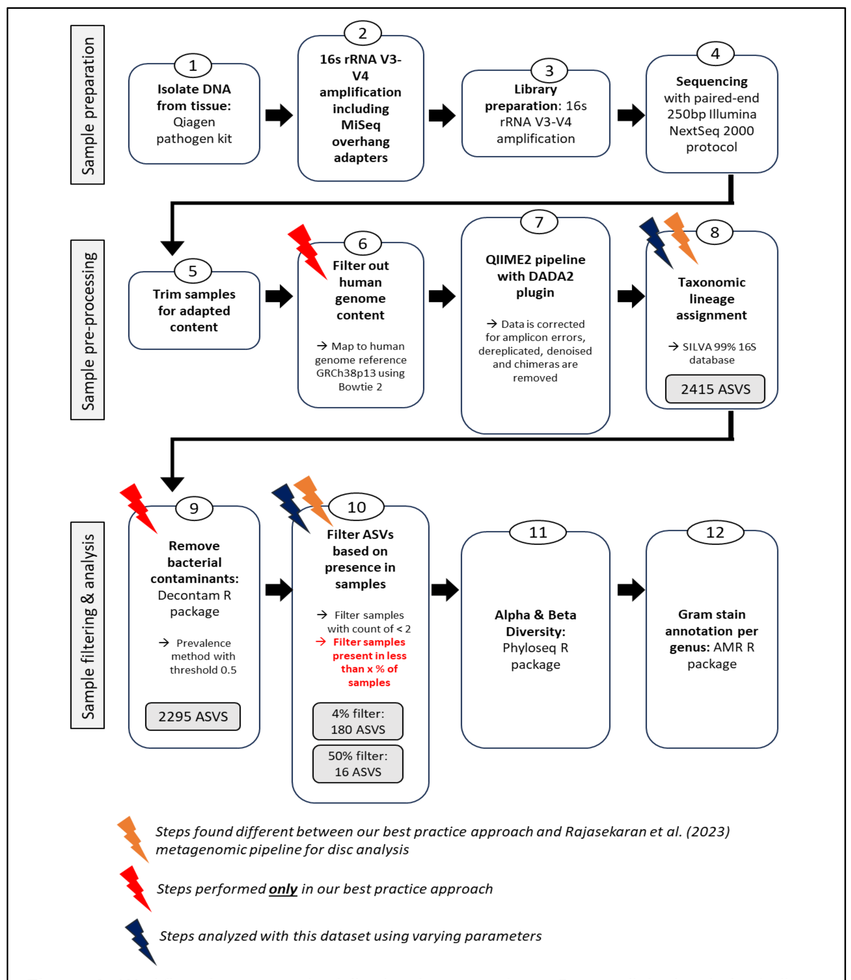

Figure 1. Workflow for our study, following best practices. Orange lightning bolts indicate variations from Rajasekaran et al.'s (2023) MC metagenome investigation, red indicates unique steps in this workflow, and blue bolts represent steps further explored using our dataset.

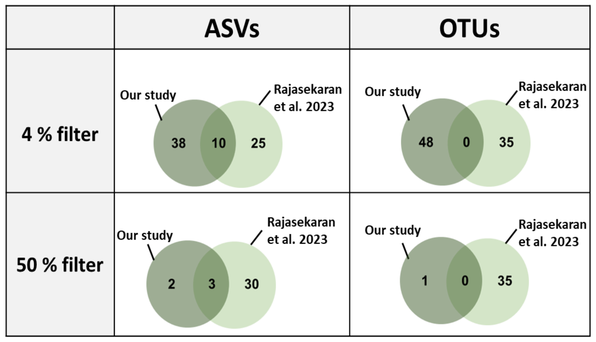

Figure 2. Direct comparison of the number of genera detected in our study compared to the number presented by Rajasekaran et al. (2023). Different parameters including the use of either ASVs or OTUs as well as the application of two different prevalence cut-off filters to our data were applied to our data.

4. Results

There was limited overlap of results with a previous study on MC disc microbiome. No bacterial genera were shared using the same bioinformatic parameters (Figure 2). Taxonomic lineage assignment using "amplicon sequencing variants" was more sensitive and detected 48 genera compared to 22 with "operational taxonomic units" (previous study) (Figure 3). Increasing filter cut-off from 4% to 50% (previous study) reduced genera from 48 to 4 genera (Figure 4). Despite these differences, both studies observed dysbiosis with an increased abundance of gram-negative bacteria in MC discs (Figure 5) as well as a lower beta-diversity. Cutibacterium was persistently detected in all groups independent of the bioinformatic approach, emphasizing its prevalence.

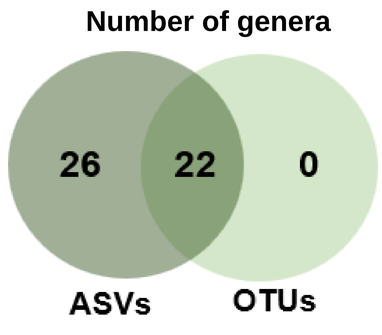

Figure 3. ASV compared to OTU annotation of genera found in at least 4 % of all samples. The overlap of genera detected with ASVs compared to OTUs.

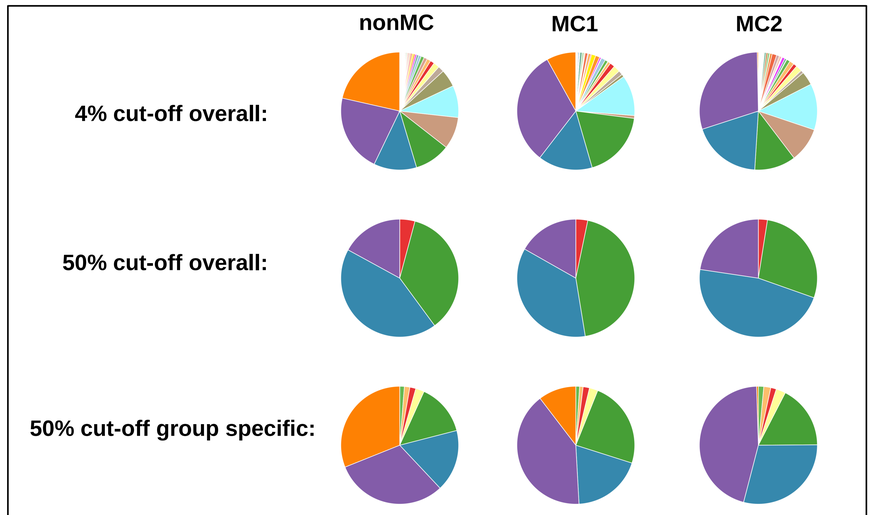

Figure 4. Comparison of different filter cut-offs. Genera distribution based on the median abundance per genera with three different filters: genera detected in more than 4 % of patients overall (top row), genera detected in more than 50 % of patients in at least one group (middle row) and genera detected in overall more than 50 % of patients (bottom row).

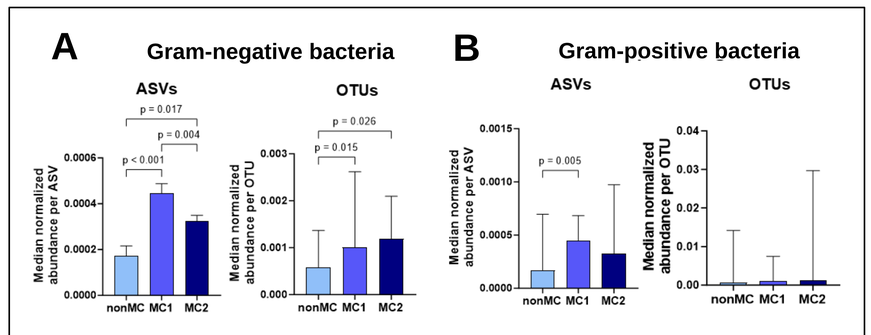

Figure 5. (A) Median abundance of **(A)** gram-negative genera or **(B)** gram-positive genera extracted with ASVs or OTUs compared between nonMC, MC1 and MC2.

5. Conclusion

There is dysbiosis in MC discs. Bioinformatic parameters impact results yet cannot explain the different findings from this and a previous study. Therefore, discrepancies are likely caused by different sample preparations or true biologic differences. Harmonized protocols are required to advance understanding of the disc microbiome and its clinical implications.