

Title: Analysis of the composition of the intestinal microbiota and its evolution in patients post-intestinal anastomosis

Protocol Code: MIDESIN

Version 2, September 19, 2025

Laboratory of New Surgical Therapies, IIS-FJD

Ethics Committee Code: PIC165-25_FJD

SUMMARY

Study Identification

Title: "Analysis of the Composition of the Intestinal Microbiota and Its Evolution in Patients Post-Intestinal Anastomosis"

Code: MIDESIN

Version: 1, July 22, 2025

Study Sites

The study will be conducted at the Hospital Universitario Fundación Jiménez Díaz and the Instituto de Investigación Sanitaria Fundación Jiménez Díaz.

Ethics Committee

The study will be submitted to the CEI/CEIm of the Hospital Universitario Fundación Jiménez Díaz. A

Ethics Committee Code: PIC165-25_FJD

Approved: 2025, 19th September

Hypothesis

Considering natural interindividual variability, the scientific literature suggests that specific microbiological patterns may exist among patients with colorectal cancer. Our primary hypothesis is that within this patient population, the colorectal microbiota differs between patients who develop postoperative anastomotic leakage and those who do not experience postoperative complications.

Objectives

Primary Objective

To characterize the microbiome of patients undergoing colorectal surgery and evaluate the impact of current surgical preparation on the microbiota of the local population.

Secondary Objectives

- Classification and prediction of postoperative evolution in patients undergoing colorectal anastomosis, through metagenomic characterization of the intestinal microbiota.
- Analyze the relationship between intestinal microbiota and its potential involvement in postoperative anastomotic leaks.

Study Design

Prospective, descriptive, non-interventional research project in which some variables will be analyzed cross-sectionally.

Condition Under Study

Any type of intestinal surgery in which an intestinal anastomosis is performed.

Study Population and Sample Size

A total of 30 patients requiring colorectal anastomosis surgery and meeting the inclusion/exclusion criteria will be enrolled.

Timeline

Start: December 2025

Recruitment: 12 months

Follow-up: 1 month per patient

Total duration: 13 months (until December 2026)

Additional time: 3 months for sample analysis + 1 month for result review.

Statistical Analysis Plan

STATISTICAL ANALYSIS

Sample Size

Based on AL incidence (9–10%), n=30 deemed sufficient.

Statistical Methods

Statistical analysis of microbiota correlations and clinical evolution

All statistical comparisons will be made taking into account the grouping of patients according to their clinical evolution, for which a matrix of possible comparisons will be made based on the clinical metadata provided by researchers at the Jiménez Díaz Foundation.

Both longitudinal analyses, which will allow us to see the temporal evolution of the patients' microbiota and how surgical preparation influences it, and cross-sectional analyses will be performed, taking into account variables related to the incidence of dehiscence and the clinical evolution of each group of patients, in search of intestinal microbiota parameters that serve as biomarkers to predict suture failure after surgery.

These analyses will be performed using the R-studio statistical packages for Windows 11 and include, among others, the following packages: dplyr, ggplot2, ggpubr, pheatmap, readxl, RColorBrewer, remotes, stringi, stringr, tibble, tidyr, vegan, ANCOM-BC, Maaslin2, microbiome, microbiomeMarker, phyloseq, and Adonis.

Results will be published and made publicly available, following ethical obligations.