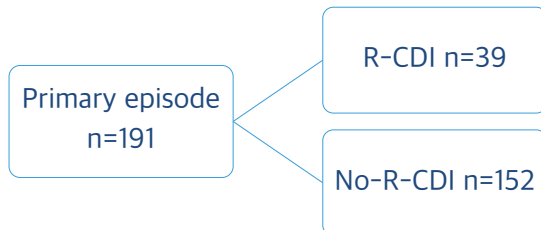


Introduction and purpose

- One of the major risk factors for *Clostridioides difficile* infection (CDI) is the gut microbiota dysbiosis.
- About 15-30% of CDI patients will have a recurrent CDI episode (R-CDI).
- Identifying risk factors or biological markers for R-CDI is important for proper treatment and prevention.
- Up to date, there are no objective markers that can predict the appearance of R-CDI.
- Calprotectin is a known biomarker of inflammatory processes.
- The objective of this study was to assess the role of the microbiome and calprotectin as biomarkers of R-CDI.

Methods



- Clinical and fecal samples were collected after informed consent was obtained.
- The hypervariable V4 region of the 16s rRNA gene was sequenced on Illumina Miseq platform according to standard protocols.
- Data preprocessing, OTU clustering and taxonomic classification were done using MOTHUR software, RDP and SILVA database.
- Alpha diversity and statistical analysis were conducted by MOTHUR and R software.
- Calprotectin levels were measured using EDITM Quantitative Fecal Calprotectin ELISA (Epitope Diagnostics, Inc, San Diego, USA).

Results

- Alpha-diversity and evenness were significantly higher in the R-CDI group ($p < 0.05$; both)
- R-CDI patients had lower abundance of *Alistipes* ($p = 0.001$), and *Akkermansia* ($p = 0.003$), and higher levels of *Bifidobacterium* ($p = 0.012$) and *Anaerostipes* ($p = 0.006$) (Figure 1).

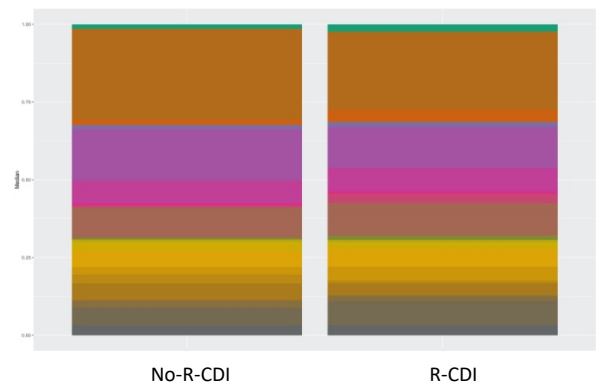


Figure 1. Different microbiota profile of patients who will develop a recurrent CDI episode (R-CDI) and who will not develop it (No-R-CDI).

- Calprotectin levels were significantly higher in R-CDI than in no-R-CDI (140.17 vs 49.05 $\mu\text{g}/\text{mg}$, $p < 0.001$) (Figure 2).

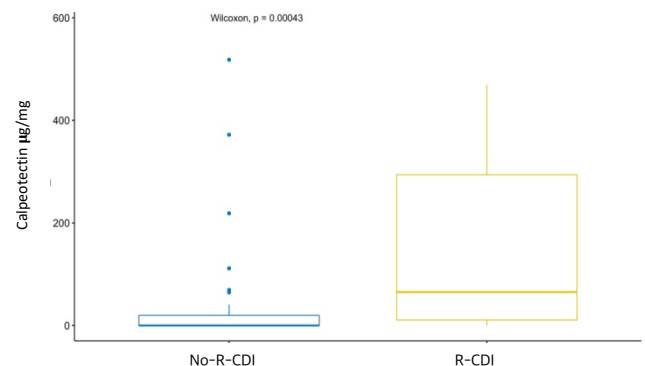


Figure 2. Calprotectin levels of patients who will develop a recurrent CDI episode (R-CDI) and who will not develop it (No-R-CDI).

Conclusions

- We identified **different microbiome profiles** at an early stage in patients who will develop R-CDI.
- We found that **high levels of calprotectin** in the primary episode were associated to developing an R-CDI episode.
- Both microbiome and calprotectin characterization in primary episodes could be helpful as early markers of R-CDI.

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