Supplementary material

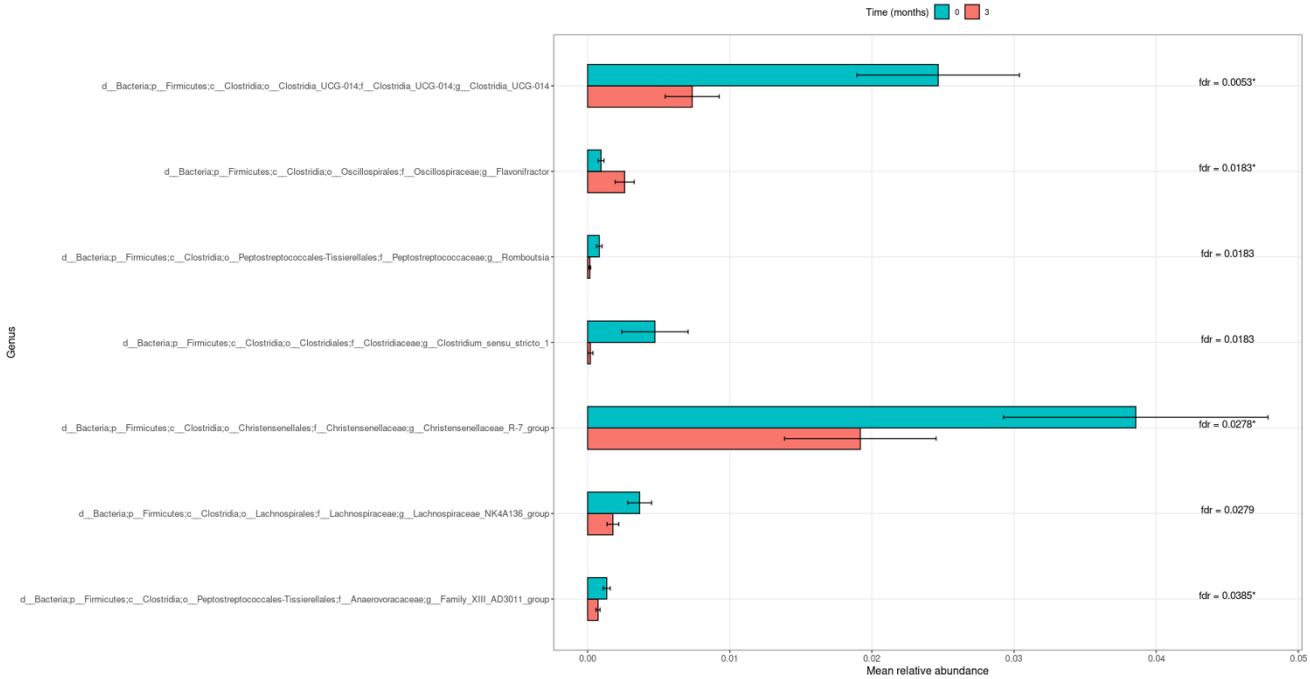
Inclusion and exclusion criteria.

1.1 Inclusion criteria
Between 18 and 75 years of age
Symptomatic heart failure, NYHA class II-III
Left ventricular ejection fraction < 40 % on echocardiography
Receiving optimal treatment for heart failure for at least 3 months
Hemoglobin > 100 g/L
Estimated glomerular filtration rate ≥ 30 mL/min
Alanine aminotransferase < 150 units/L
Signed informed consent
Acceptable acoustic windows for echocardiographic assessment

1.2 Exclusion criteria
Treatment with antibiotics or probiotics within 12 weeks prior to randomization
History of hypersensitivity to rifaximin or other rifamycin derived antimicrobial agents, or any of the components of Xifaxan®
History of hypersensitivity to *S boulardii*, yeast, or any of the components of Precosa ®
Polypharmacy with increased risk for interactions, i.e., an extensive list of medications (e.g. 10 drugs or more) which may influence with the patient safety or compromise the study results
Malignancy of any cause, excluding basal cell carcinoma of the skin, which has not been curatively treated > 5 years ago, or where there has been relapse within the last 5 years
Acute coronary syndrome within 12 weeks prior to randomization
Cardiac resynchronization therapy during the past six months

NYHA class = New York Heart Association functional classification

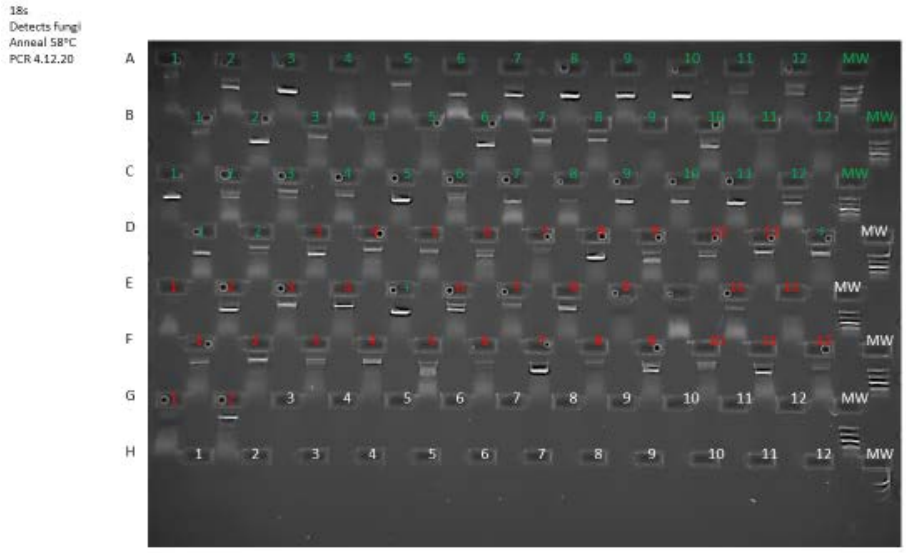
Bar plot at genus level for the rifaximin group at baseline compared to three months.



P-values are FDR corrected. * indicates, in addition, a statistical significant difference as compared to the standard of care group.

Isolation of Saccharomyces cerevisiae genomic DNA and PCR.

Genomic DNA of 500 µl overnight culture of INVSc1 strain was isolated according to standard protocol. (1) Conventional PCR was performed to assess presence of fungal genes in isolated stool DNA. Following primers were used: ACT1: 3` ACGTTGGTGATGAAGCTCAA `5, 3` AGCAGTGGTGGAGAAAGAGTA `5 designed using Primer3 tool on the SGD yeastgenome webpage (2); universal fungal 18s B2F and B4R primers: 3` ACTTTCGATCGTAGGATAG `5, 3`TGATCGTCTTCGATCCCCTA `5 and D1 and D2 for delta element amplification 3` CAAAATTCACCTATWTCTCA `5, 3` GTGGATTTTTATTCCAACA `5. (3, 4) PCR conditions: Phusion HF master mix 1x (Thermo Scientific), 400 nM final primer concentration, 3% DMSO, 30ng genomic DNA, total volume of 20 µl. **Cycling conditions:** Hot start at 98ºC 1 min followed by 34 cycles of 98ºC 20 sec, 58ºC (ACT1/18s)/54ºC (D1/D2) and 72ºC 50 sec. Final extension at 72ºC for 5 min.



Samples marked in green were randomized to *saccharomyces boulardii*, whereas samples marked in red were not. Samples marked with + were *saccharomyces boulardii* controls, while minus are negative controls.

Safety and monitoring

Definitions

Adverse events (AE)
An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.
An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. The term AE is used to include both serious and non-serious AEs.
If an abnormal laboratory value/vital sign are associated with clinical signs and symptoms, the sign/symptom should be reported as an AE.

Serious Adverse Event (SAE)
Any untoward medical occurrence that at any dose:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

Expected Adverse Events
The expected AEs for Rifaximin:
• Most common adverse reactions in travellers' diarrhoea (≥ 5%):
Flatulence, headache, abdominal pain, rectal tenesmus, defecation urgency and nausea
• Most common adverse reactions in Hepatic Encephalopathy (≥ 10%): Peripheral oedema,
nausea, dizziness, fatigue, ascites, flatulence, and headache
Although no common AEs are reported for Precosa, less common AEs (<1%) include allergic reactions, urticaria, constipation and thirst. Fungemia and sepsis have occurred in severely ill patients.

References

1. Dymond JS**.** Preparation of genomic DNA from Saccharomyces cerevisiae. Methods Enzymol. 2013;529:153-60.
2. Yeastgenome.org. 2020. Primer3 | SGD. .
3. Imre A, Rácz HV, Antunovics Z, Rádai Z, Kovács R, Lopandic K, et al. A new, rapid multiplex PCR method identifies frequent probiotic origin among clinical Saccharomyces isolates. Microbiol Res. 2019;227:126298.
4. Stefanini I, Albanese D, Sordo M, Legras J-L, De Filippo C, Cavalieri D, et al. SaccharomycesIDentifier, SID: strain-level analysis of Saccharomyces cerevisiae populations by using microsatellite meta-patterns. Scientific Reports. 2017;7(1):15343.