# The identification of prospective prognostic markers and probiotics in the gut microbiome for the protection of the vulnerable endemic population from diarrhea

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...meter of socio-economic loss globally and is a recurring threat in the endemic regions of the world, specially in the low-income countries (LICs) and lower-...quencing can help to avert diarrhea and can help to assuage disease burden.

...5 diarrheal fecal samples was conducted in Kolkata to identify statistically significant antagonistic interactions among communities of commensals and ...markers and probiotic candidates.

...volunteers after informed consent. 16s rRNA amplicon sequencing on the Illumina MiSeq platform and subsequent analysis using the QIIME pipeline was ...of gut microbiome structural diversity between the diarrheal and non-diarrheal gut microbiome was analyzed and statistically evaluated to understand the significance of the differences in the context of community interaction in the gut microbiome under normobiosis and dysbiosis.

**Results:** Significant differences between the two groups with respect to the structural composition was revealed. Firmicutes was the most abundant phylum in the majority of the samples. B/F ratio was consistently <1 in all diarrheal samples. Significant difference in mean B/F ratio of the two groups was found. Proteobacteria was significantly more abundant in the diarrheal group. Prevotellaceae was the most abundant family in non-diarrheal samples and was suppressed significantly in diarrheal samples. *Streptococcaceae* was the most abundant family in 60% diarrheal samples and where *Streptococcaceae* was suppressed, Bacteroideaceae and Nocardeaceae were the most abundant. In non-diarrheal samples where *Streptococcaceae* was almost completely suppressed Bifidobacteriaceae was the most ... and a significant negative correlation was observed between *Prevotellaceae* and *Bacteroideaceae* in the non-diarrheal group. *Prevotella copri* was the most abundant species in 70% non-diarrheal samples and w... ... in all the non-diarrheal samples while they were absent in diarrheal samples.

**Discussion:** The OTUs associated with diar... ...comparative analysis of diarrheal and non-diarrheal microbiome, to our knowledge, and distinctly addressing the gut microbiome dysbiosis from the context that can lead to t... ...population from diarrhea.

**Conclusion:** This study highlighting signific... ...conclusively determined with the help of metagenomics and statistical analysis that the diarrheal gut microbiome undergoes depletion of beneficial commensal bacteria ... ...c and probiotic candidates can be further developed to establish normobiosis in the gut of the endemic population vulnerable to diarrhea and these can be administered in t...

## 1. Introduction

- Diarrhea is the 9th major cause of mort... ...ren contributing to 5,25000 deaths annually globally, accounting for 9% of u... ...the highest incidence and mortality and include countries in Africa, South-East Asia and Latin America many of wh... ...2019 6,32,344 deaths were recorded for all ages and 55,309 deaths were recorded for under 5 children.
- It is a major cause of malnutrition. Maln...
- Diarrhea is preventable through hygiene... ...y practices
- ORS, 20mg zinc and antibiotics are the... ...ever, MDR pathogens have challenged the efficiency of antibiotics and are contributing towards enhanced mortality...
- Our study was designed to work in acco... with WHO's GAPPD (Global Action Plan for Pneumonia and Diarrhea) that stipulated "Protect, Prevent and Treat" ;NGS has emerged as a potent tool in molecular epidemiology to help to fulfill these goals. It can be employed to help in the identification of potential markers for prognosis of diarrhea in endemic regions and for probiotics that can augment nutrition and be administered as dietary supplements and can also be used for treatment.

GBD Diarrhoeal Diseases Collaborators. *Lancet Infect Dis* 2017 Jun 1
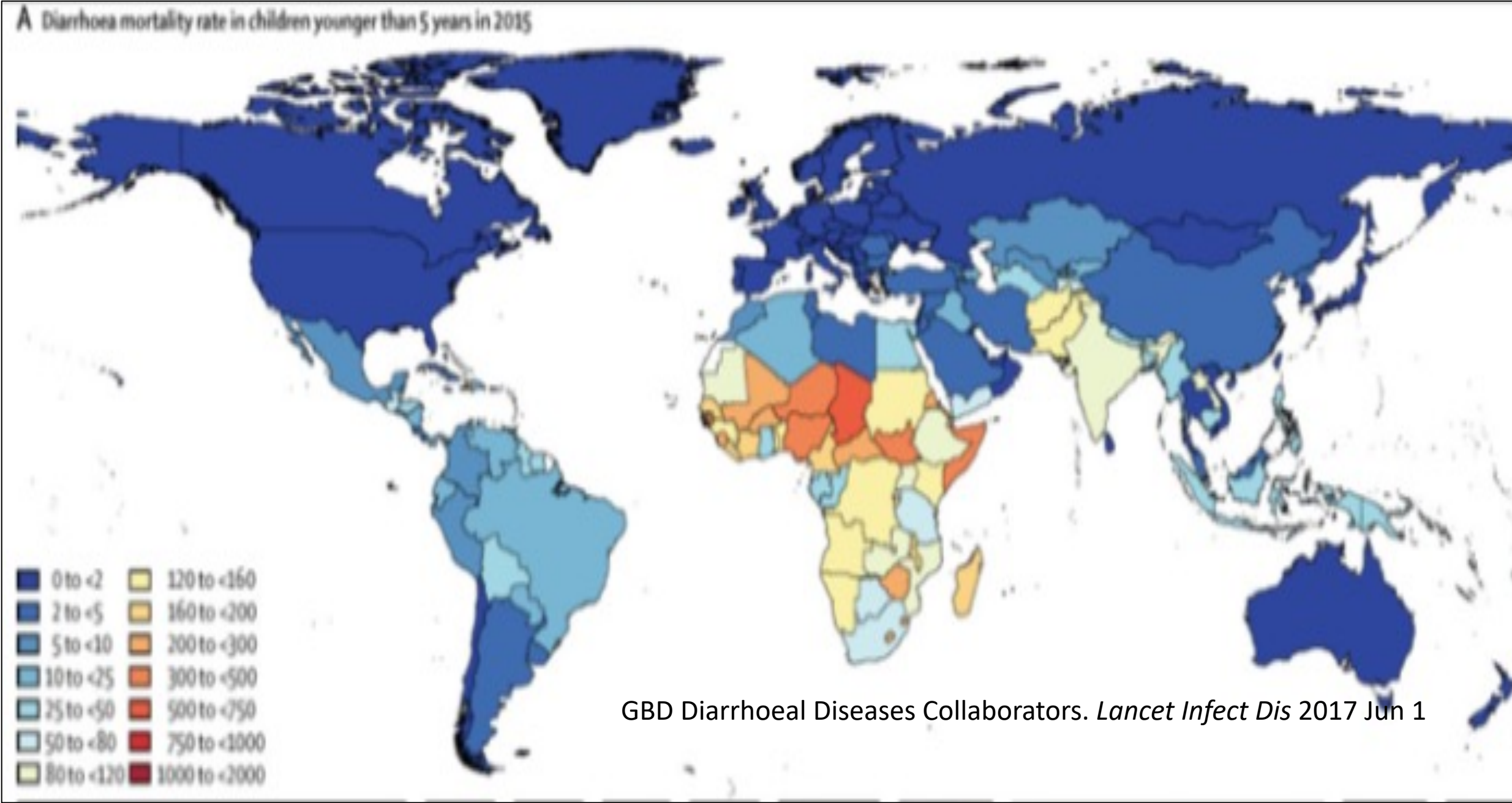
## 2. Objectives

The objective of the study was to examine dysbiosis in diarrhea and identify taxa that can be used as prognostic and therapeutic markers to revert dysfunctional gut by restoring normobiosis:

1. Differential identification of diversity and abundance of gut microbiota taxa in diarrheal and non-diarrheal population
2. Identify taxa associated with diarrheal dysbiosis
3. Statistically analyze antagonistic community interactions between commensal and pathobiont flora in the two groups-diarrheal and non-diarrheal

## 3. Method

- A cross-sectional gut microbiome analysis of 23 non-diarrheal and 5 diarrheal fecal samples was conducted in Kolkata after informed consent. GITC method was used for DNA extraction.16S rRNA amplicon sequencing (V3-V4 regions) on the Illumina MiSeq platform was performed and analysis was done using the QIIME pipeline
- The query sequences were clustered using the UCLUST method against the Greengene database. Taxonomies were assigned at >=97% sequence similarity. Downstream analysis and visualization was performed using R-package. Relative abundance from phylum to species was calculated from read counts assigned to OTU divided by total utilized reads for microbiome search and was presented as stacked column plot. B/F ratio was calculated. The difference in mean relative abundance was calculated for the major phyla and families and the results were presented using stacked bar-diagram. The significance of this difference was calculated with students' two-tailed T-test and correlation with the help of Pearson correlation coefficient. Z score was used to analyze difference of proportion of the two groups with respect to a taxon.

## 4. ...

- ... ...el ... ...nicutes, Bacteroidetes were prese... ...c ... ...ance of the major phyla and the... ...ce ... ...e been presented in Figure 1. Fir... ...in ... ...eal and 52.2% non-diarrheal samples with a mean abundance... ...42% in non-diarrheal. Bacteroidetes were dominant in 43.5% n... ...in none of the diarrheal samples. The mean abundance of Bacteroidetes was 7.72% in diarrheal and 41.71% in non-diarrheal (control) samples. The difference was significant with *p*-value 0.000114. The average B/F ratio in diarrheal samples was 0.23 and was consistently <1 in all the diarrheal samples while in non- diarrheal samples the average was 1.23 with 11 out of 23 samples having a B/F ratio >1. B/F ratios is significantly different with a *p*-value of 0.008228. The mean abundance of Proteobacteria was 21.76% in diarrheal and only 3% in non-diarrheal samples and the difference was significant with *p*-value< .00001. Phyla TM7 and Fusobacteria were significantly higher in diarrheal samples compared to non-diarrheal samples.

- Significant differences in abundance was observed for *Lachnospiraceae*, *Erysipelotrichaceae*, *Prevotellaceae*, *Streptococcaceae*, *Enterobacteriaceae*, *Vellionellaceae* and *Ruminococcaceae*. *Prevotellaceae* was the most abundant family in 69.6% non-diarrheal samples and was suppressed significantly in diarrheal samples while *Streptococcaceae* was the most abundant family in 60% diarrheal samples. Both the differences in frequency of occurrence in the two groups expressed by Z-score was significant with p-values 0.004 and 0.00008 respectively. In diarrheal samples in which *Streptococcaceae* was suppressed, *Bacteroideaceae* and *Nocardeaceae* were the most abundant. In non-diarrheal samples where *Streptococcaceae* was almost completely suppressed *Bifidobacteriaceae* was the most abundant and suppressed other families significantly. A negative correlation was observed between *Prevotellaceae* and *Bacteroideaceae* in the control. In diarrheal samples negative correlation of *Prevotellaceae* and *Lachnospiraceae* was found with *Streptococcaceae* and *Enterobacteriaceae* while *Ruminococcaceae* was found to have negative correlation with *Enterobacteriaceae*. In non-diarrheal samples significant negative correlation was found between *Lachnospiraceae* and *Enterobacteriaceae*.

- *Proteus mirabilis* was found associated with all non-diarrheal samples while it was absent in diarrheal samples. *Prevotella copri* was found in all the samples of both the groups though the mean abundance in diarrheal samples was 14.86% while in non-diarrheal samples was 22.21% and the difference was significant with *p*-value .012063.
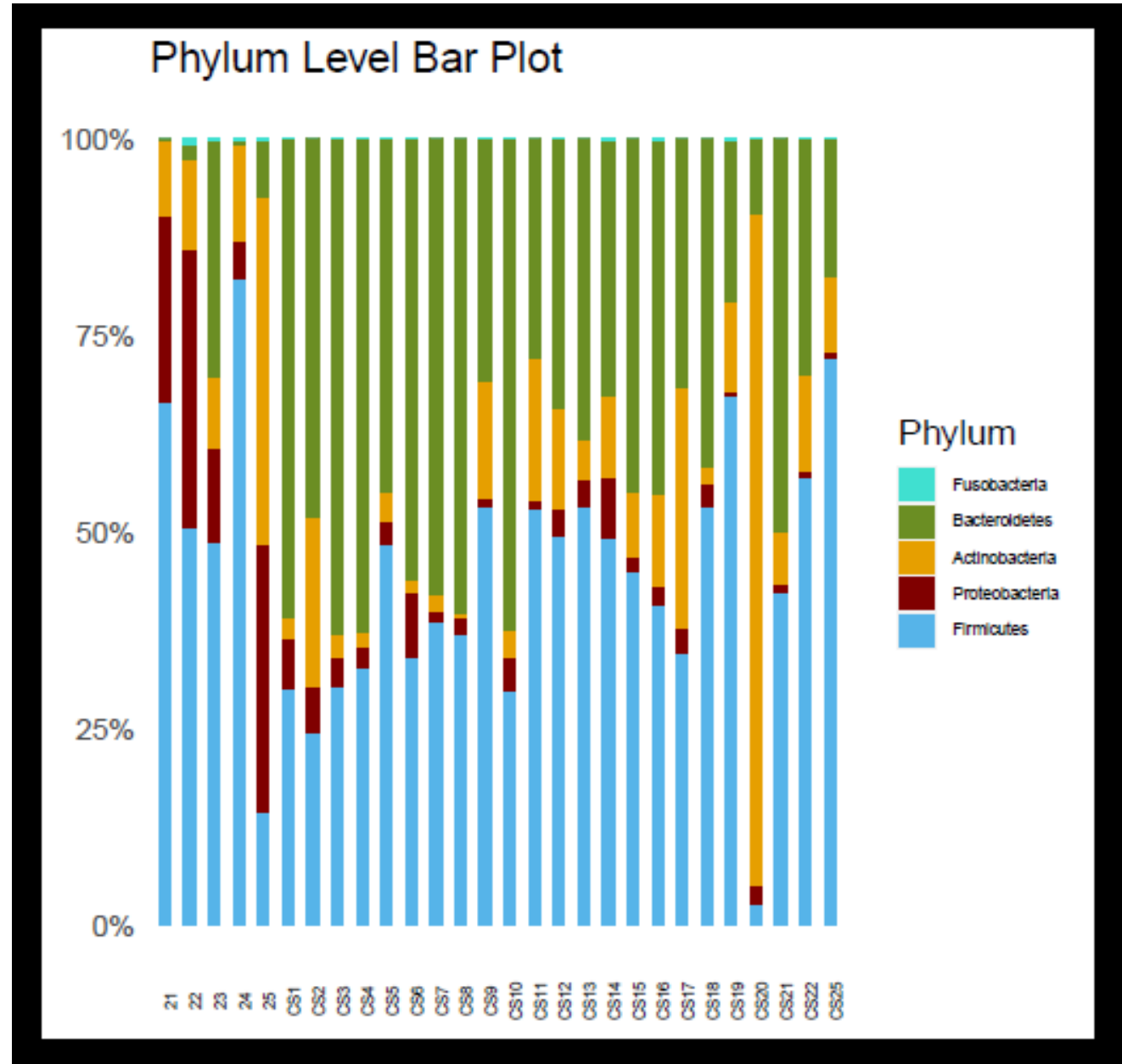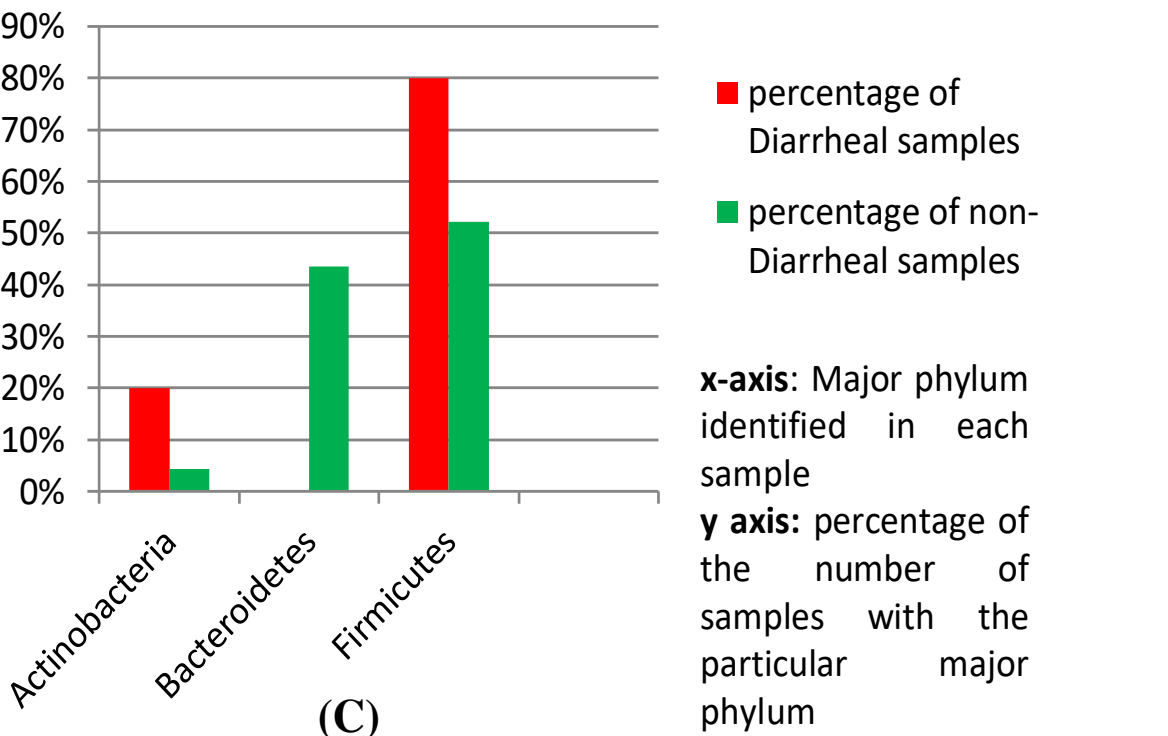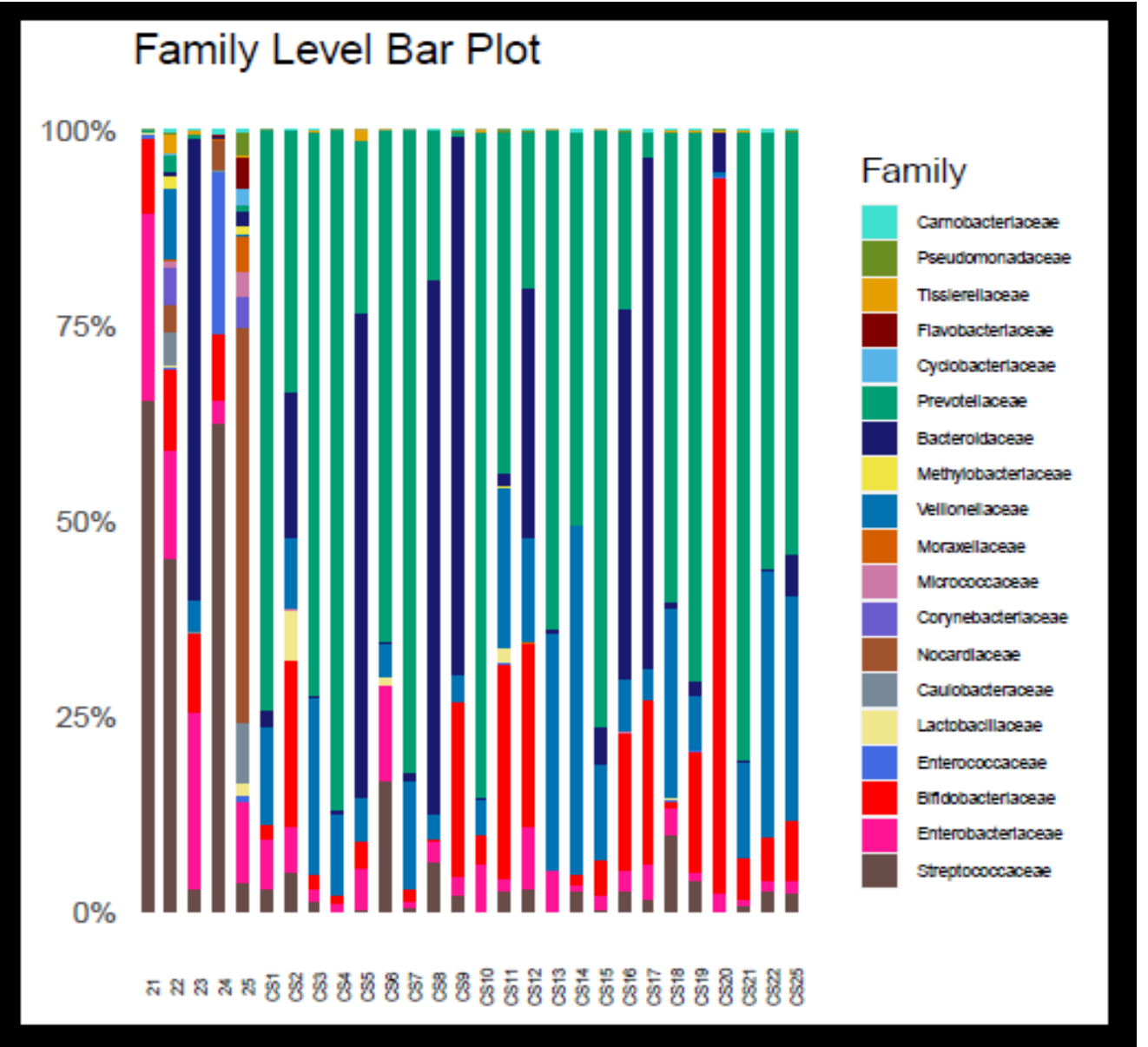
**Figure 1** showing (A) the relative abundance of and (B) difference in relative abundance of the major phyla in 5 diarrheal samples 21-25 and non-diarrheal samples CS1-CS22 and CS25(C) shows the frequency of finding Actinobacteria, Firmicutes, Bacteroidetes as the most dominant phylum in the population expressed as percentage of diarrheal and non-diarrheal samples carrying the particular phylum
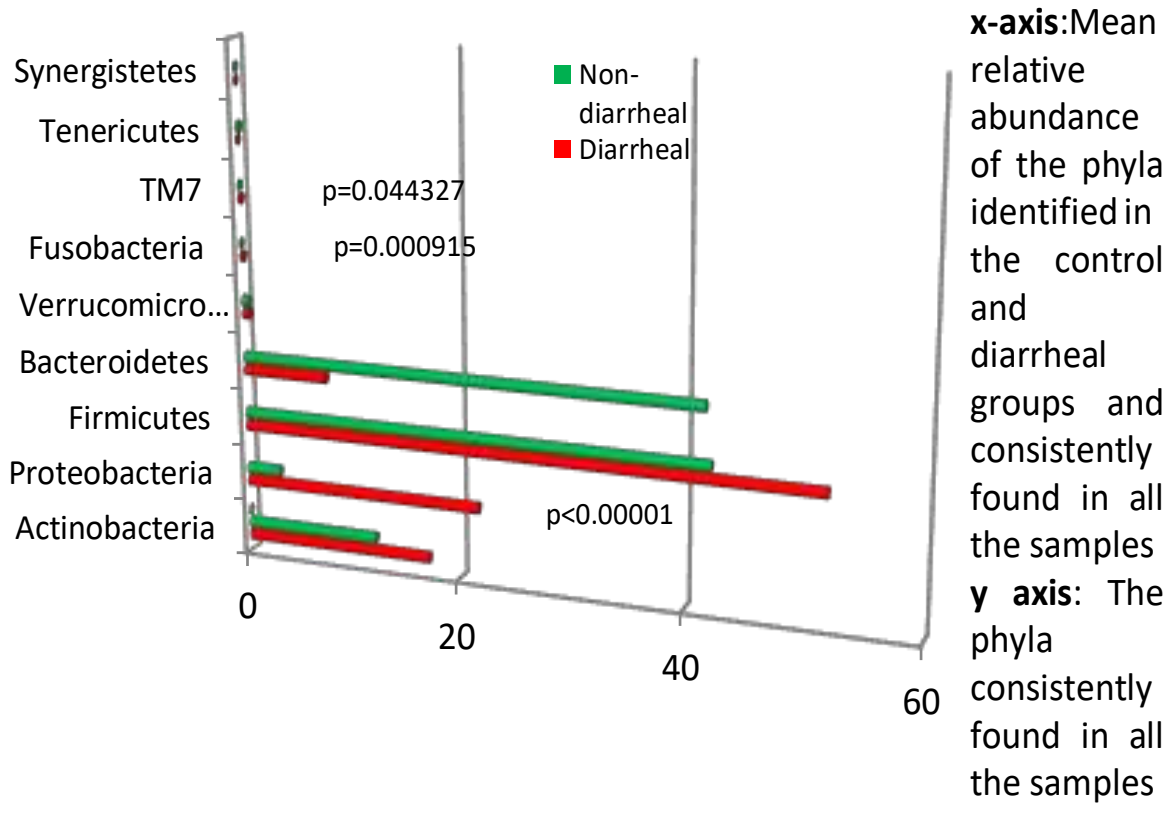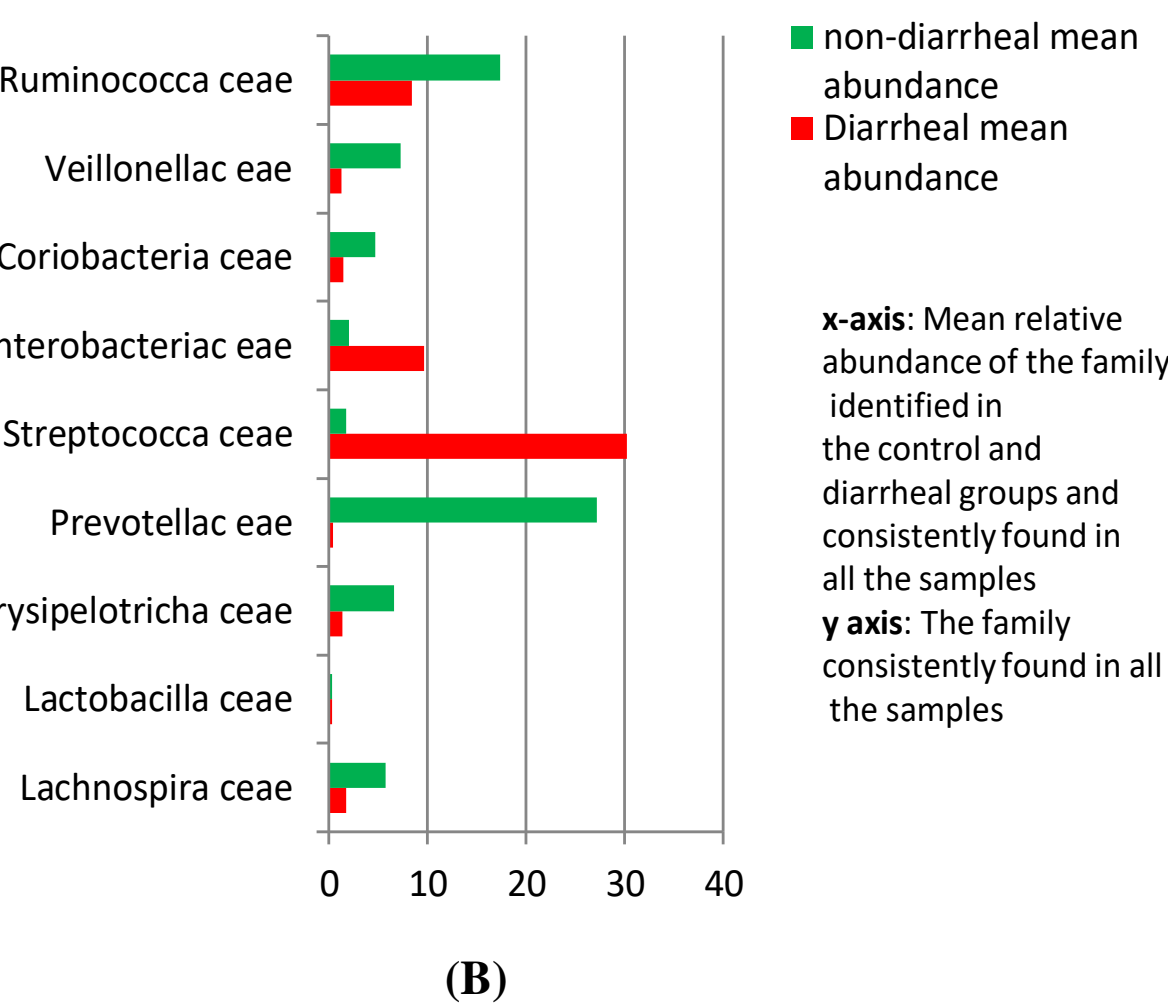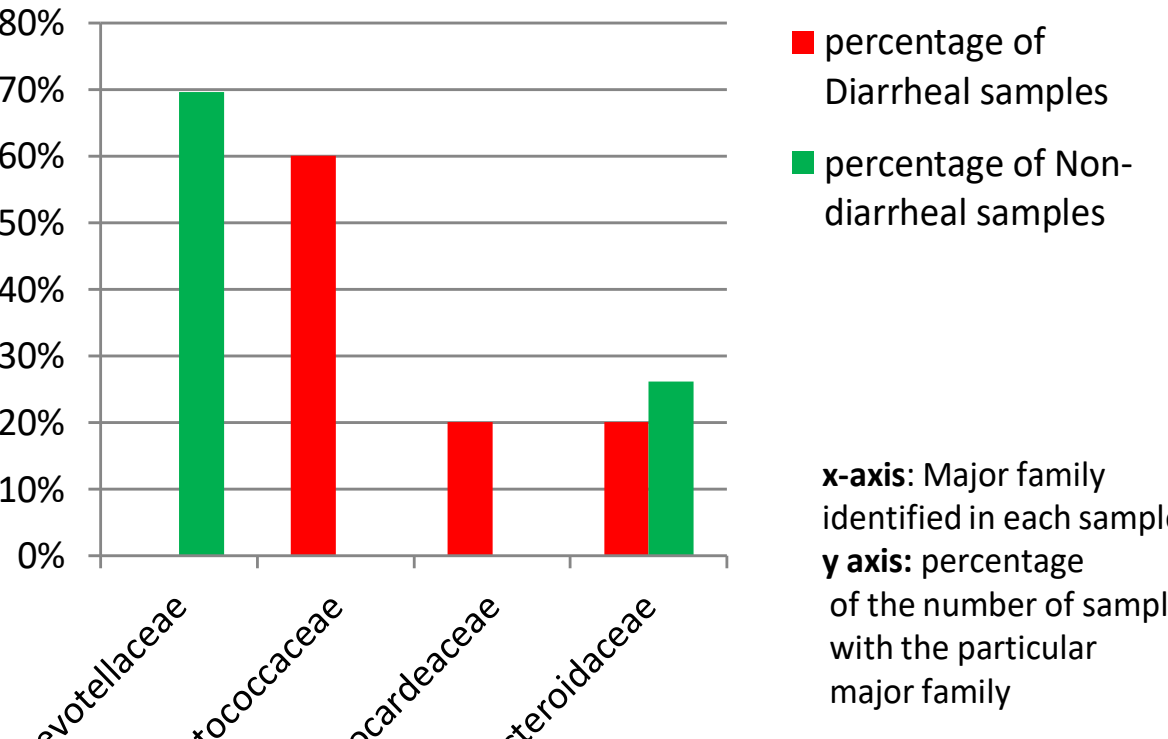
**Figure 2** showing (A) the of the relative abundance of and (B) difference in relative abundance of the major families in 5 diarrheal samples 21-25 and non-diarrheal samples CS1-CS22 and CS25 (C) shows the frequency of finding *Prevotellaceae*, *Streptococcaceae*, *Nocardeaceae*, *Bacteroidaceae* as the most dominant family in the population expressed as percentage of diarrheal and non-diarrheal samples carrying the particular family
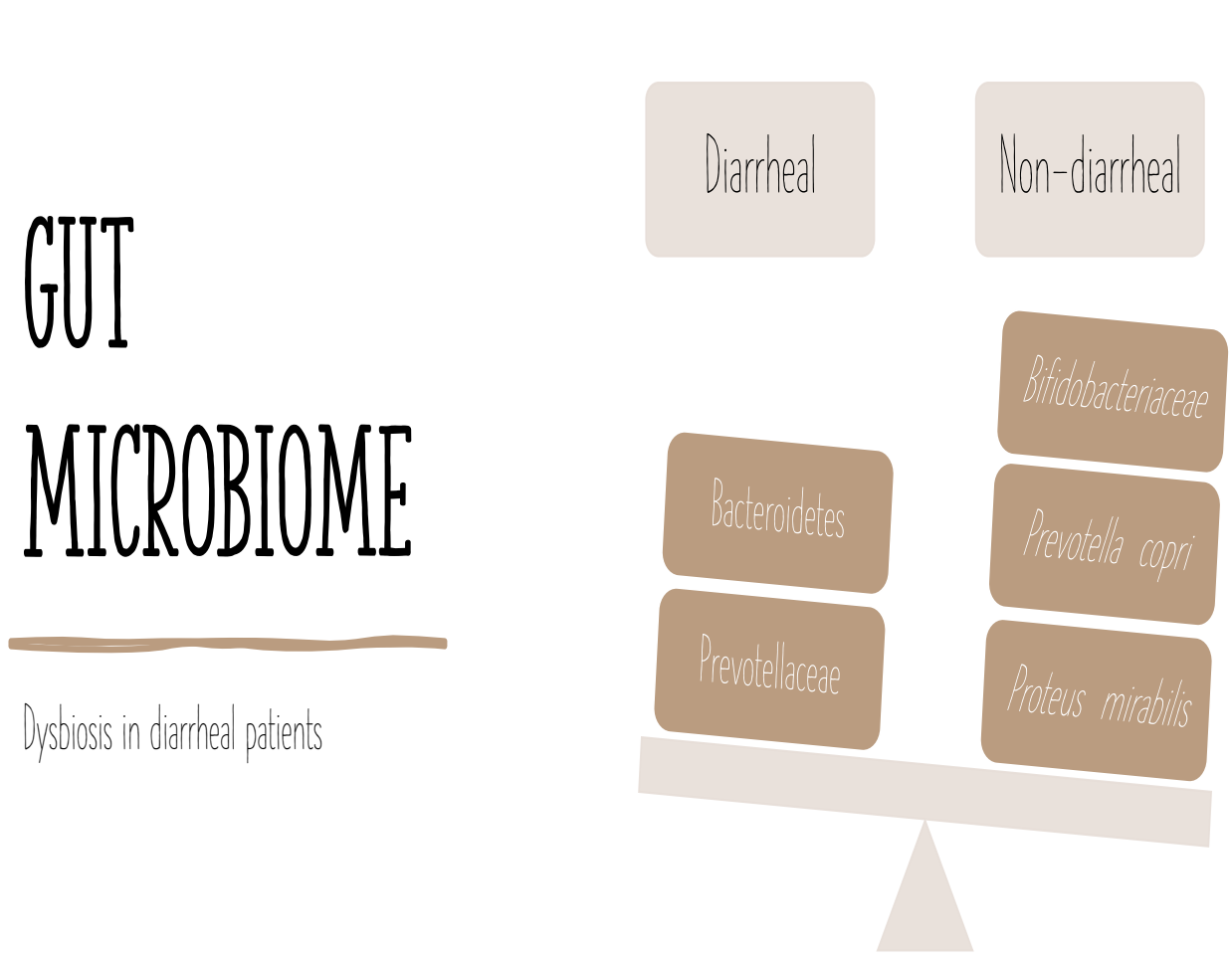
## 5. Discussion

A negative correlation of commensals and pathobionts among the 5 diarrheal samples was found. In the non-diarrheal microbiota significant negative correlation occurs between commensal *Lachnospiraceae* and *Enterobacteriaceae*. This could serve as a prognostic marker for screening vulnerability to diarrhea. *Bifidobacteriaceae* is dominant over pathobionts.

In the non-diarrheal group, *Prevotellaceae* was significantly enriched and a significantly higher abundance of *P. copri* was observed and can serve as a marker of diarrheal dysbiosis.

The findings were reinforced by epidemiological data that the frequency of occurrence of these markers were significantly different in the two groups.

These may be developed as probiotics for the endemic population to promote normobiosis and prevent diarrhea. *P. copri* has been already found to be a potential next-generation probiotic. Another interesting feature revealed by our study was the total absence of *P. mirabilis* in diarrheal patients.

## 6.Conclusion

This is the first report on the comparative analysis of diarrheal and non-diarrheal gut microbiome. We identified prospective OTUs which can serve as potential prognostic markers and can also be developed into prospective probiotics to provide protection to the endemic population. This would benefit, particularly, the economically backward areas of the world where gut microbiome dysbiosis due to different parameters like malnourishment make them vulnerable to diarrheal pathogens. These could be administered as dietary supplements to accord normobiosis to protect from diarrhea and also as therapeutics. This would help in the attainment of the Sustainable Development Goals, SDGs 2 and 3 of the United Nations.

## 7. Key messages:

1. New age technology like next-generation sequencing is a highly potent tool for the detection, prediction and prospective development of prognostic and therapeutic markers.

2. The gut microbiome holds tremendous prospects for the development of novel probiotics and prognostic markers.

3. Our research methodology is unique and novel and highly prospective for understanding the gut microbiome from a critical vantage point that has led to the identification of prognostic and probiotic candidates that can help to revert dysbiosis associated with diarrhea and establish normobiosis in the gut microbiome. These candidates may be administered as dietary supplements to accord protection to the endemic population vulnerable to diarrhea.

## 6. References:

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