Microbiota signatures of under nutrition: gut microbiota and under nutrition in India

G. Balakrish Nair, PhD

Communicable Diseases Department

South East Asia Regional Office

World Health Organisation
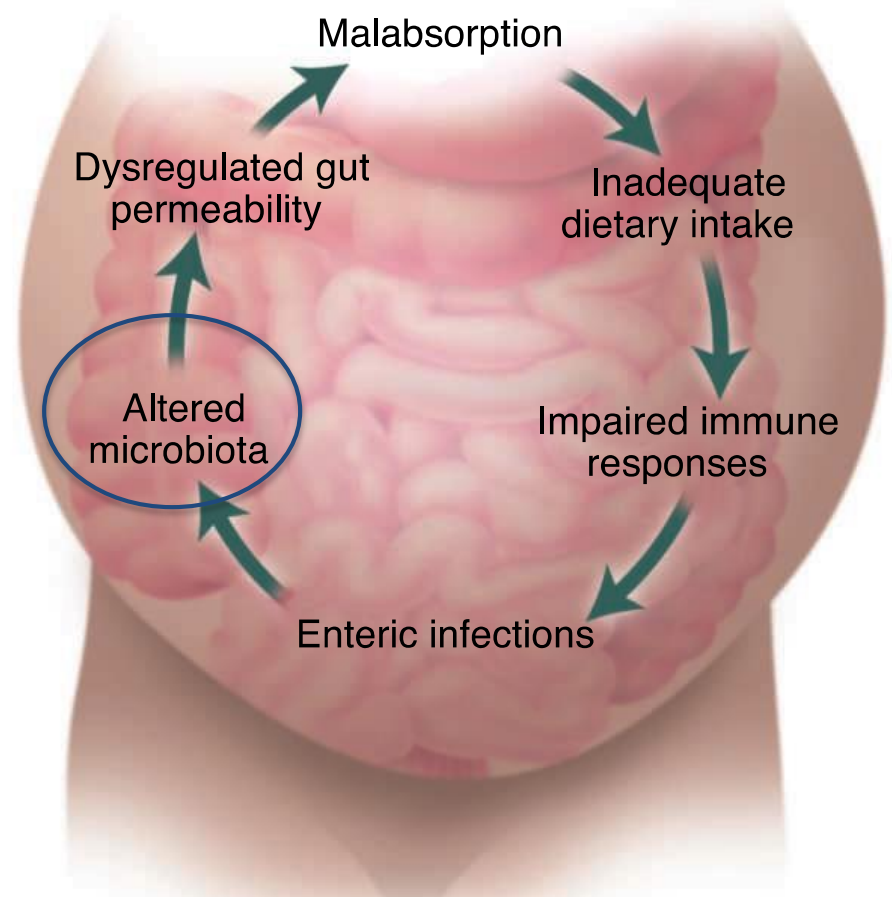
New Delhi, India

nairg@who.int

Presentation Outline

❑ Undernutrition – Definition and Responsible factors

❑ Global statistics on under nutrition with special emphasis on India

❑ Gut Microbiota and Under nutrition : the Malawian Twin study and Bangladeshi study

❑ The Indian Study

❑ Key Messages

What is Undernutrition?

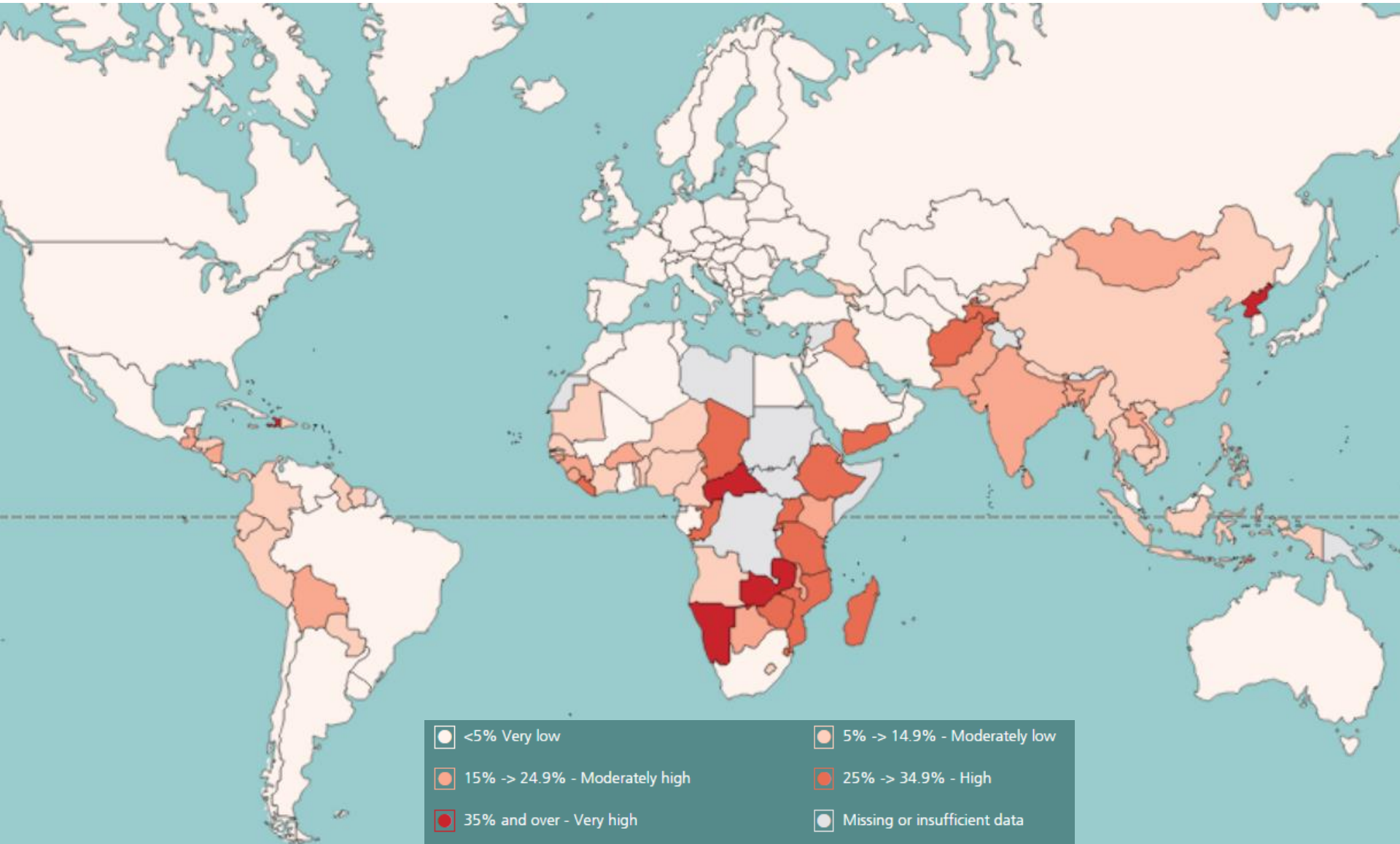**Undernutrition** is **defined** as the outcome of insufficient food intake and repeated infectious diseases.

Worsens and perpetuates each other as a result of impaired innate and adaptive immune responses.

Vicious cycle of undernutrition

- Malabsorption
- Dysregulated gut permeability
- Altered microbiota
- Inadequate dietary intake
- Impaired immune responses
- Enteric infections

Relman, D.A. 2013 Science; 339:530-532

Hunger Map – the global under nutrition status
About 793 million people are under nourished globally

|	
○ <5% Very low	○ 5% -> 14.9% - Moderately low
○ 15% -> 24.9% - Moderately high	○ 25% -> 34.9% - High
○ 35% and over - Very high	○ Missing or insufficient data

Percentage of children under age 5 who are moderately or severely wasted

Ranked by burden (2011)	Country	Year	Wasting (%, moderate or severe)	Wasting (%, severe)	Number of wasted children, 2011 (moderate or severe, thousands)
1	India	2005–2006	20	6	25,461
2	Nigeria	2008	14	7	3,783
3	Pakistan	2011	15	6	3,339
4	Indonesia	2010	13	6	2,820
5	Bangladesh	2011	16	4	2,251
6	China	2010	3	–	1,891
7	Ethiopia	2011	10	3	1,156
8	Democratic Republic of the Congo	2010	9	3	1,024
9	Sudan	2010	16	5	817
10	Philippines	2008*	7	–	769

*Data differ from the standard definition or refer to only part of a country.
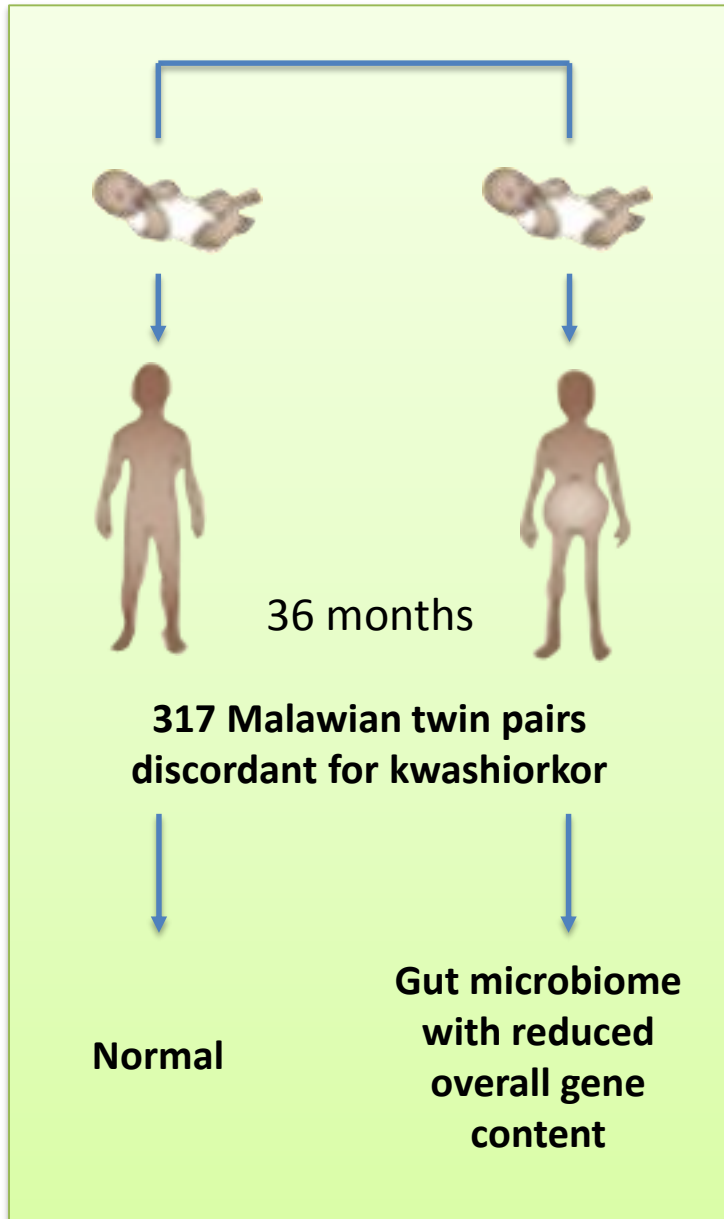
Source: UNICEF Global Nutrition Database, 2012, based on MICS, DHS and other national surveys, 2007–2011, except for India.

The vicious cycle of diarrhoea and undernutrition in susceptible children
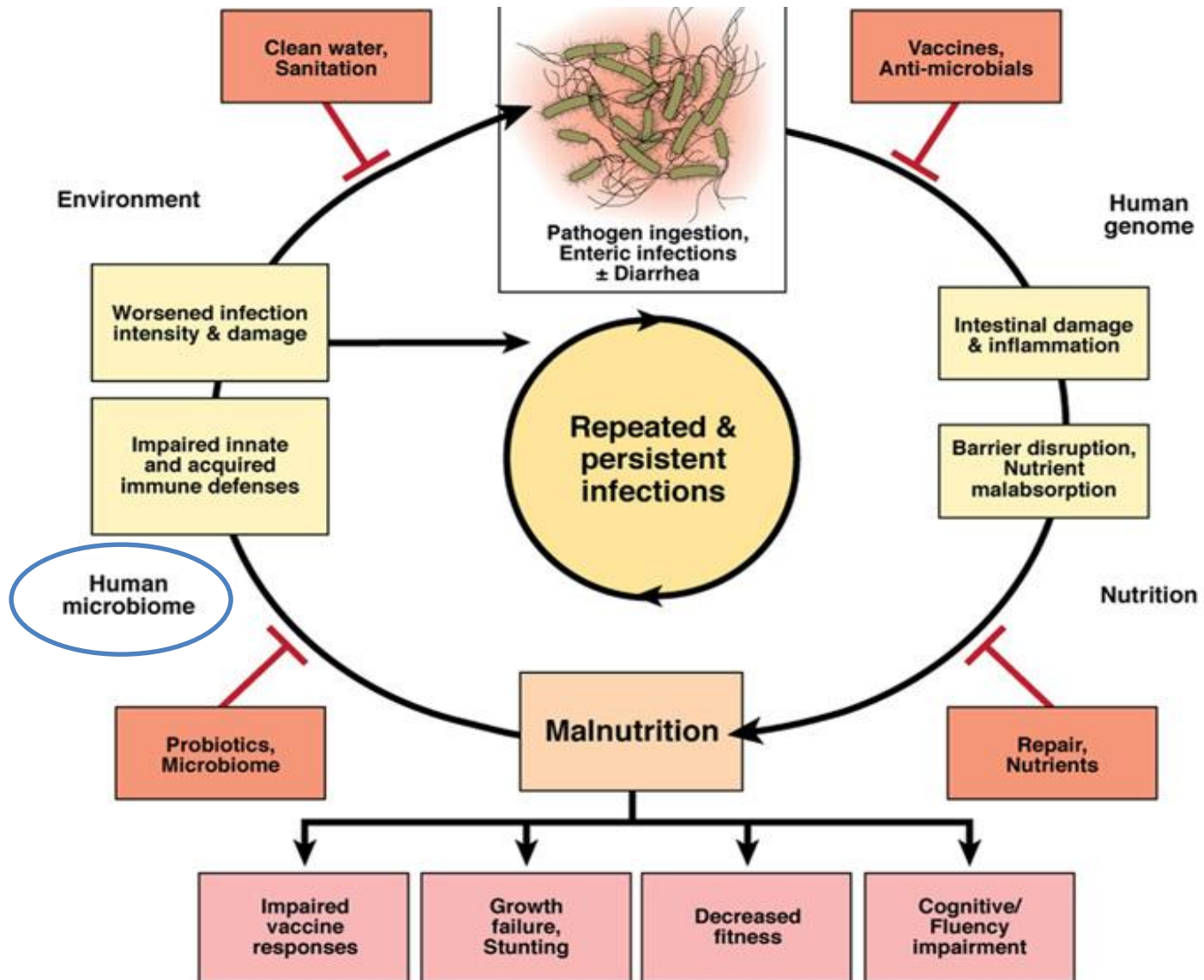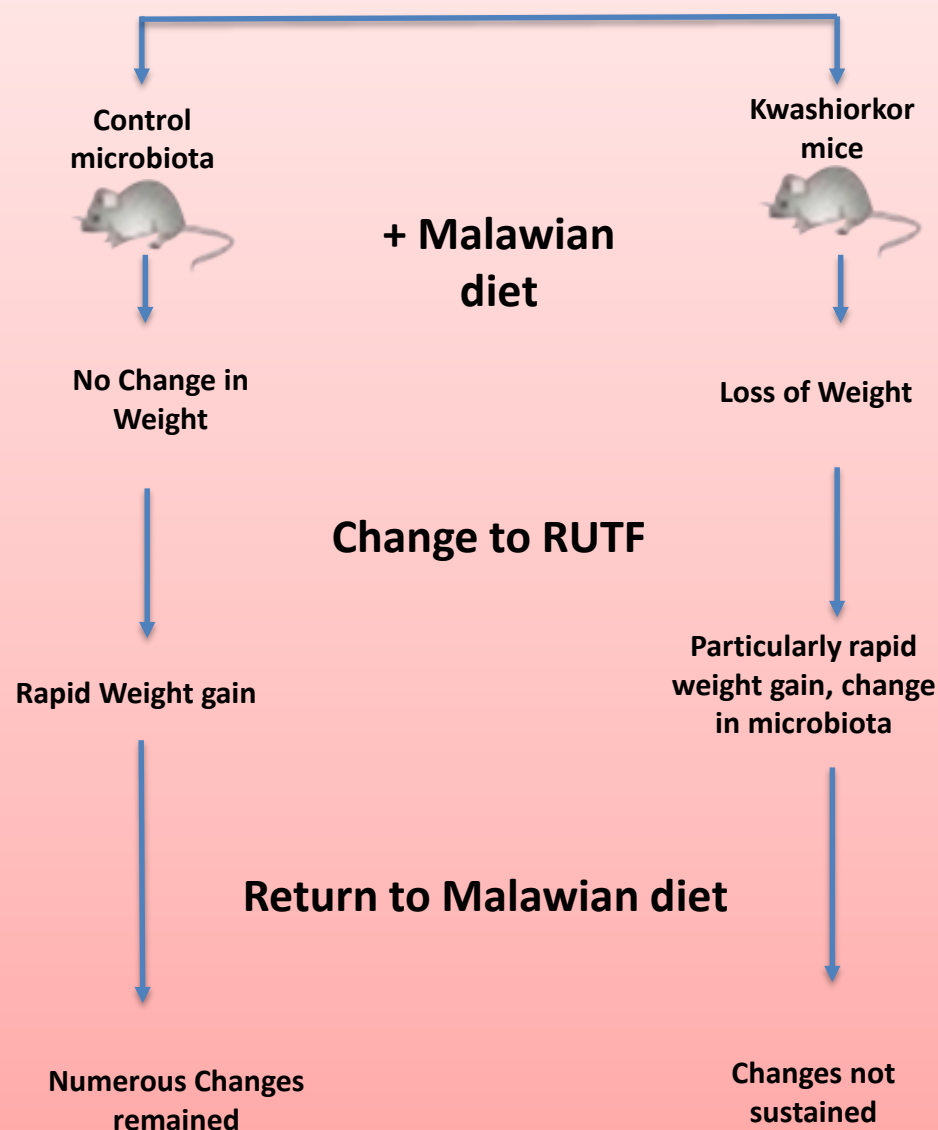
Undernutrition and Gut Microbiota – The Link

Causal relationship between gut microbiota and under nutrition

36 months

317 Malawian twin pairs discordant for kwashiorkor

Normal

Gut microbiome with reduced overall gene content

❑ Over the first two years, babies acquire a complex gut ecosystem that increases in diversity

❑ Healthy twins had a considerably more differentiated gut microbiome than those with kwashiorkor

❑ The divergent trajectory could only be transiently corrected by RUTF treatment

❑ RUTF not only improved the patients' symptoms but also had a major effect on the gut microbiota

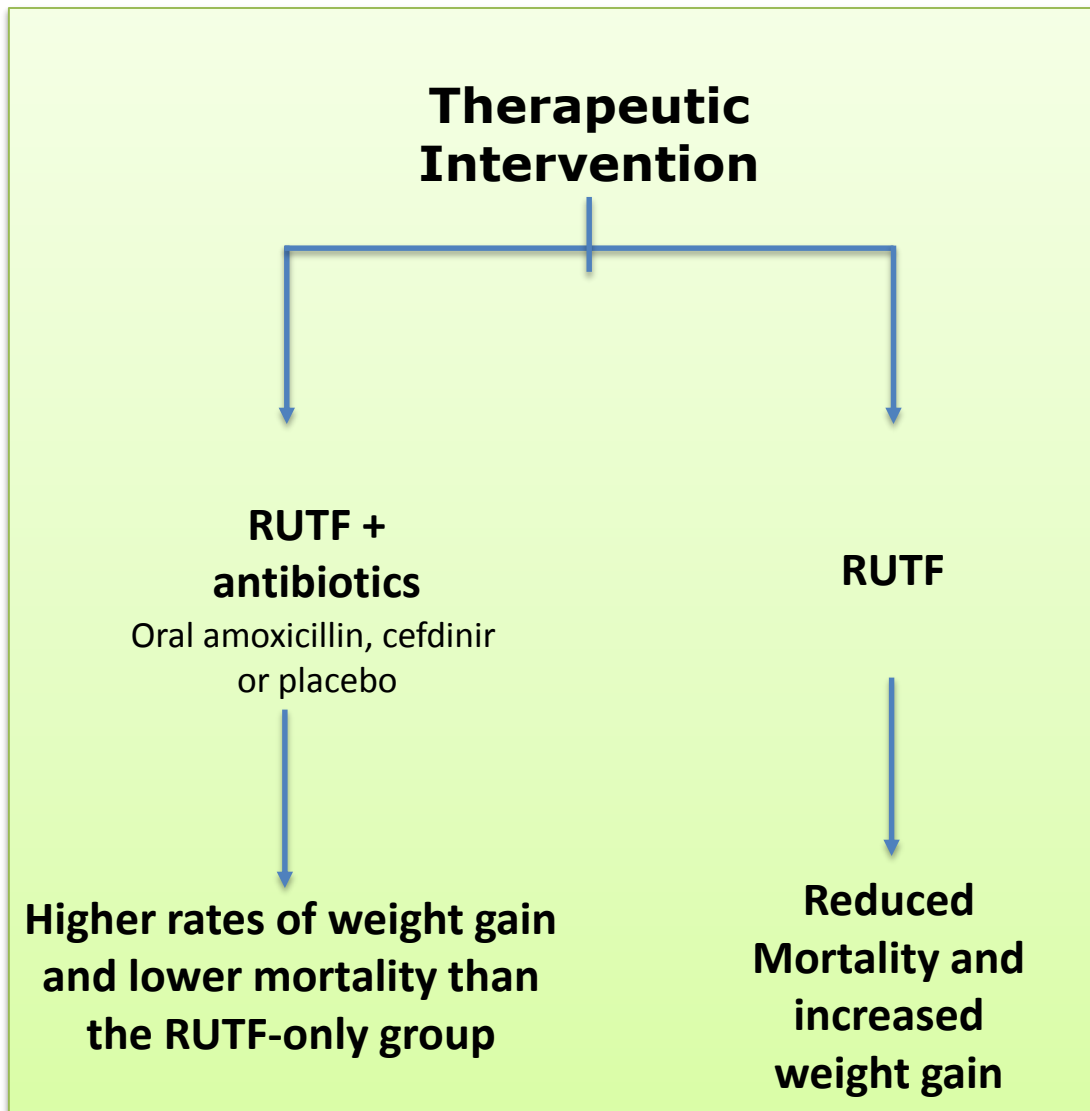❑ Could not identify a specific microbial signature characteristic of kwashiorkor infants

Smith et al. (2013) Science 339, 548-554
Tilg and Moschen (2013) G&H 261-262

Testing the causal relationship between the gut microbiome and the host metabolism

Control microbiota

Kwashiorkor mice

+ Malawian diet

No Change in Weight

Loss of Weight

Change to RUTF

Rapid Weight gain

Particularly rapid weight gain, change in microbiota

Return to Malawian diet

Numerous Changes remained

Changes not sustained

- ❑ Only mice with the combination kwashiorkor microbiota and Malawian diet lost weight.

- ❑ Changes in the gut microbiome of mice with K-like symptoms that could have contributed to manifestation of the disease

- ❑ Pathogens could not cause the discordant weight loss

- ❑ Number of bacteria that were differentially found in healthy and kwashiorkor recipient mice

- ❑ *Bilophilia wadworthia*

Smith et al. (2013) Science 339, 548-55
Tilg and Moschen (2013) G&H 261-262

Malnutrition, Microbiota and Antibiotics

Therapeutic Intervention

RUTF + antibiotics
Oral amoxicillin, cefdinir or placebo

RUTF

Higher rates of weight gain and lower mortality than the RUTF-only group

Reduced Mortality and increased weight gain

- RCCT including 2,767 Malawian children with Kwashiorkor, marasmus or marasmic Kwashiorkor investigated
- Recovery rates for the three groups 88.7%, 90.9% and 85.1%
- Mortality rates for the three groups 4.8%, 4.1% and 7.4%
- Clinical trial concluded that the addition of antibiotics to RUTF for uncomplicated SAM was associated with a considerable improvement in recovery and mortality rates

Trehan et al 2013 New Engl J Med 368:, 435-435

The Bangladesh Study
Relative abundance of the dominant bacterial phyla in the gut of healthy and malnourished children
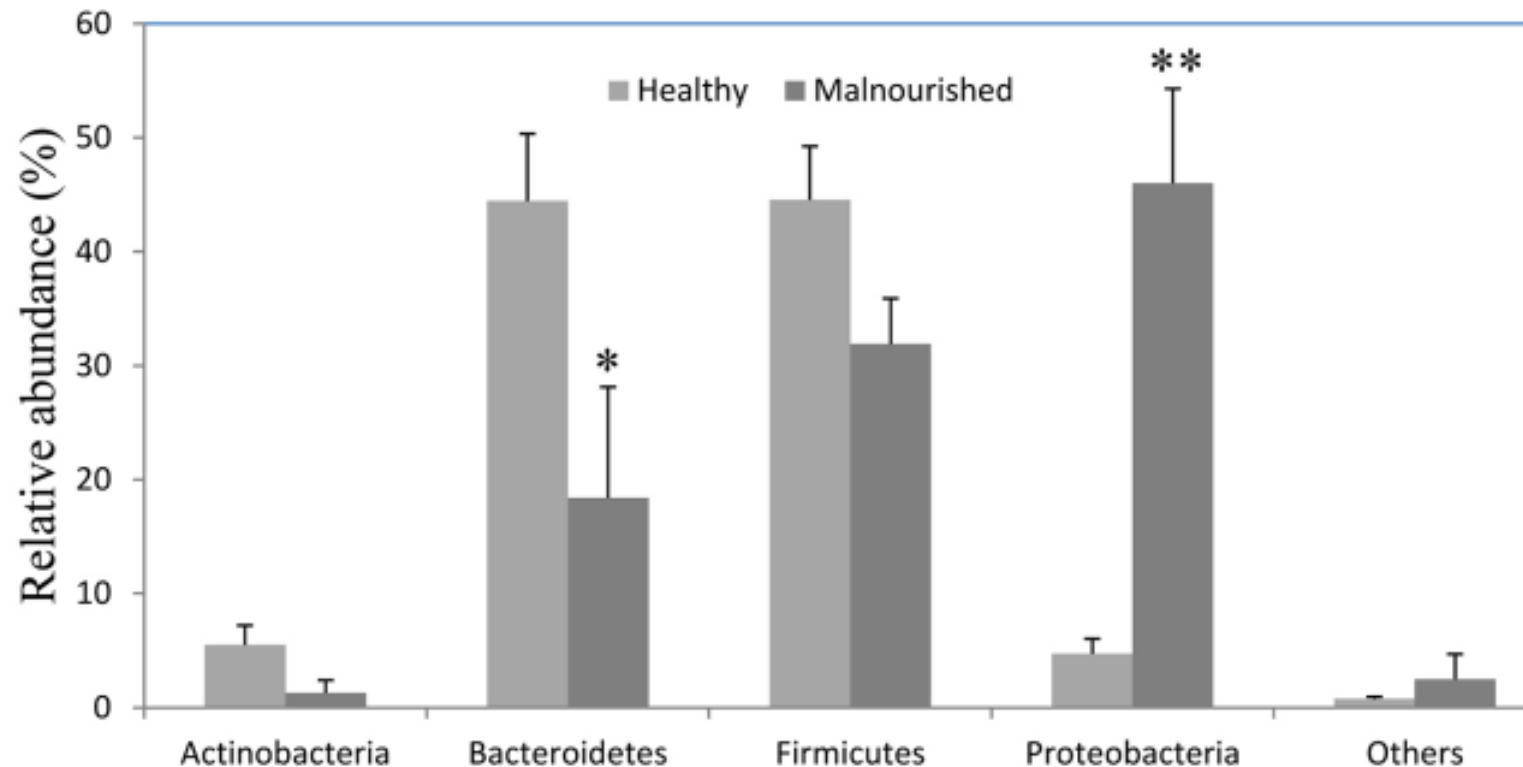
The predominance of potentially pathogenic Proteobacteria and minimal level of Bacteriodetes as commensal microbiota might be associated with the ill health of malnourished children in Bangladesh

Monira et al. 2011 Frontiers in Microbiology
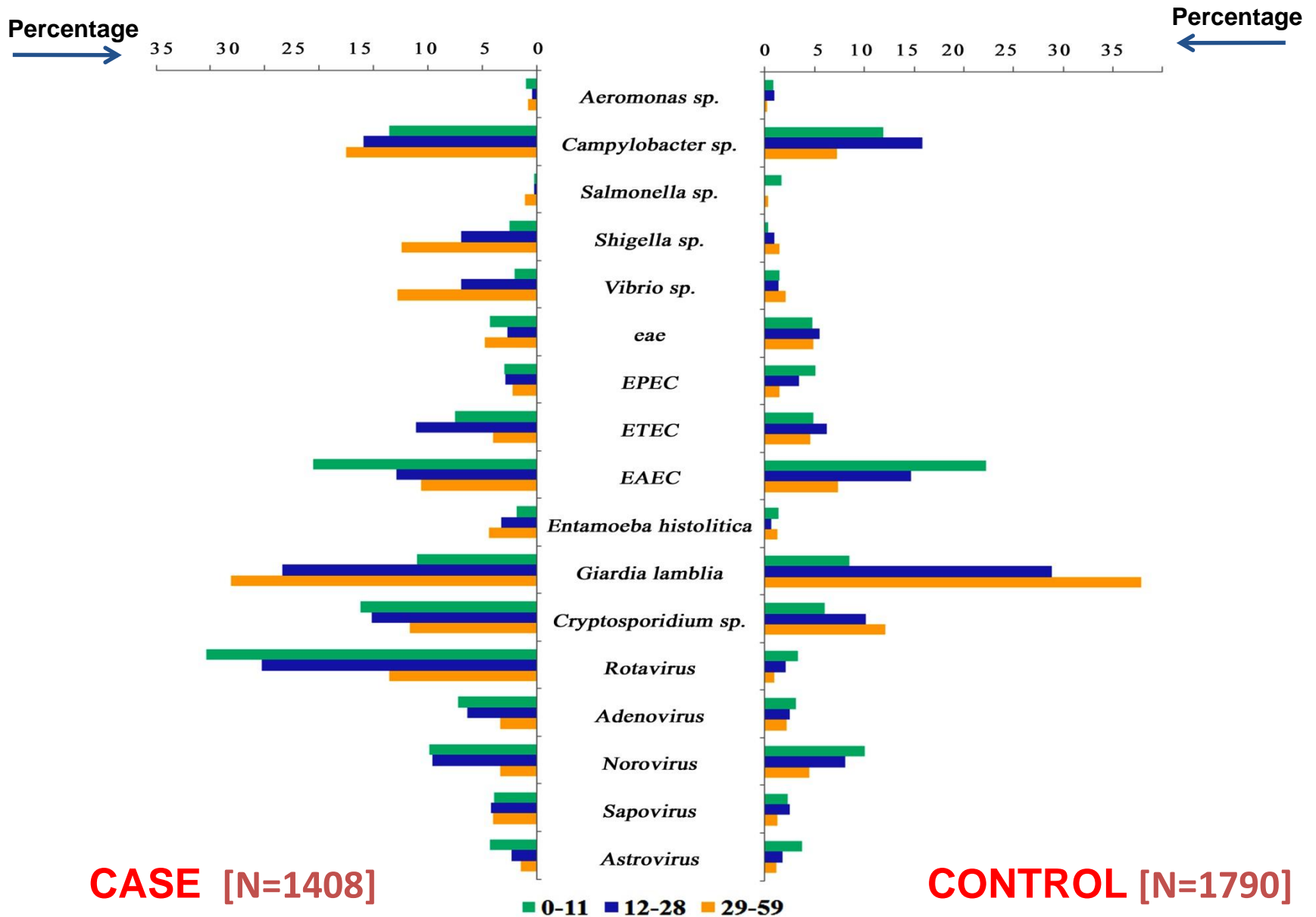
The Indian Study

Diarrhoea at the Infectious Diseases Hospital, Kolkata

November 2007 and October 2009 (Nair et al., Gut Pathogens)

Patients Admitted (45,004)

Patient Enrolled 2,536 (5.6%)

Samples taken 2,519 (99.3%)

Samples not taken 17 (0.7%)

Sole Pathogen 1,080 (42.9%)

Mixed Pathogen 736 (29.2%)

No Pathogen 703 (27.9%)

2 pathogens 528 (71.7%)

3 pathogens 155 (21.1%)

4 pathogens 41 (5.6%)

5 pathogens 8 (1.1%)

6 pathogens 4 (0.5%)
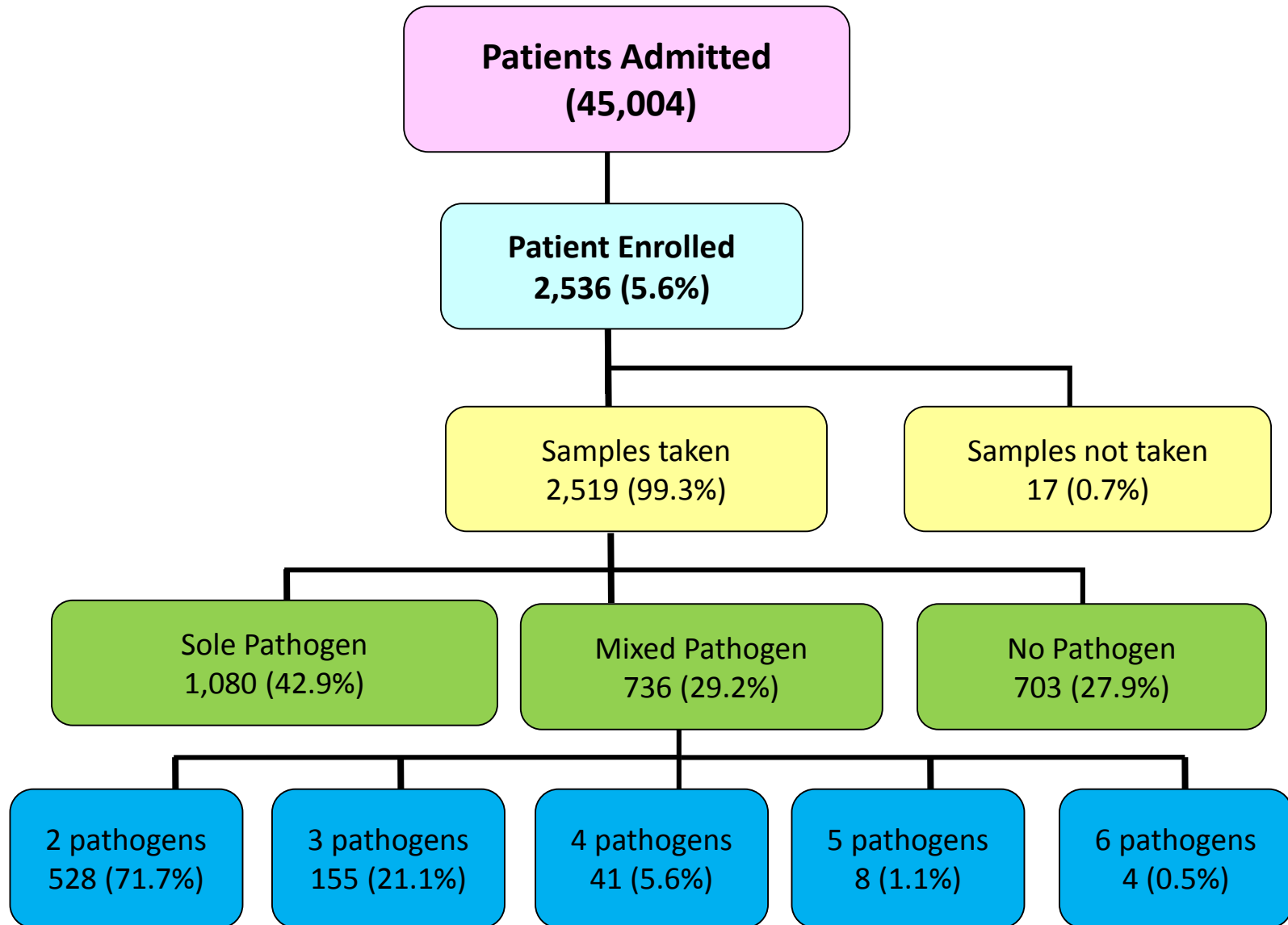
Community Diarrhoea in an urban slum in Kolkata

Global Enterics Multicenter Study

Percentage

Percentage

CASE [N=1408]

CONTROL [N=1790]

■ 0-11 ■ 12-28 ■ 29-59

ERI (%) of causative organism for diarrhoea

ERI (%) = Difference of isolation rate (%) between cases and controls
i.e. Excess rate (%) of isolation attributable to diarrhoea

Global Enterics Multicenter Study

PLOS | ONE
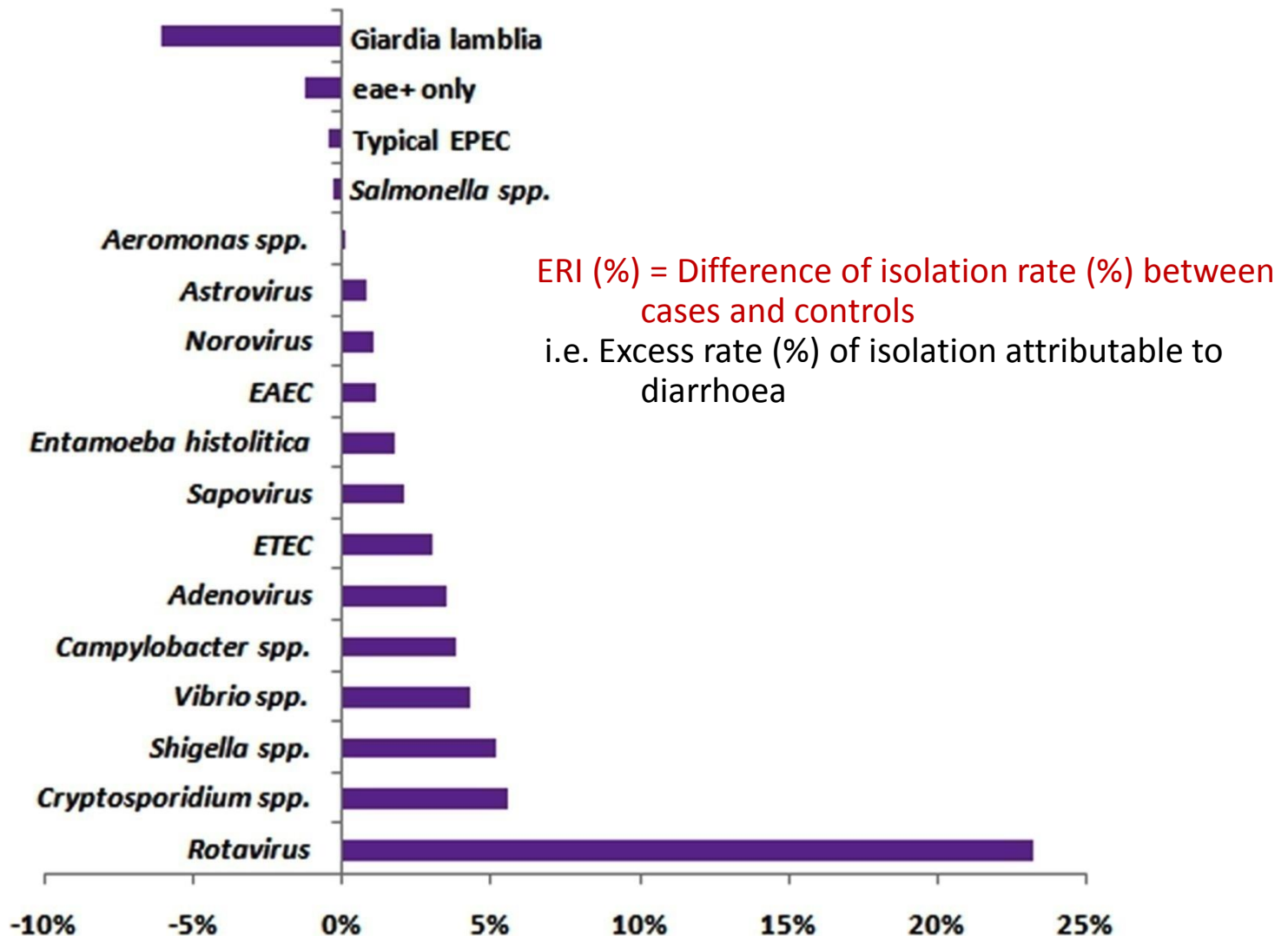
Gut Microbiomes of Indian Children of Varying Nutritional Status

Tarini Shankar Ghosh[1], Sourav Sen Gupta[2], Tanudeep Bhattacharya[1], Deepak Yadav[1], Anamitra Barik[3], Abhijit Chowdhury[3,4], Bhabatosh Das[2], Sharmila S. Mande[1]*, G. Balakrish Nair[2]*

1 Bio Sciences R&D, TCS Innovation Labs, Pune, India, 2 Center for Human Microbial Ecology, Translational Health Science and Technology Institute, Gurgaon, India, 3 Society for Health and Demographic Surveillance, West Bengal, India, 4 Department of Hepatology, School of Digestive and Liver Diseases, Institute of Post Graduate Medical Education and Research, Kolkata, India
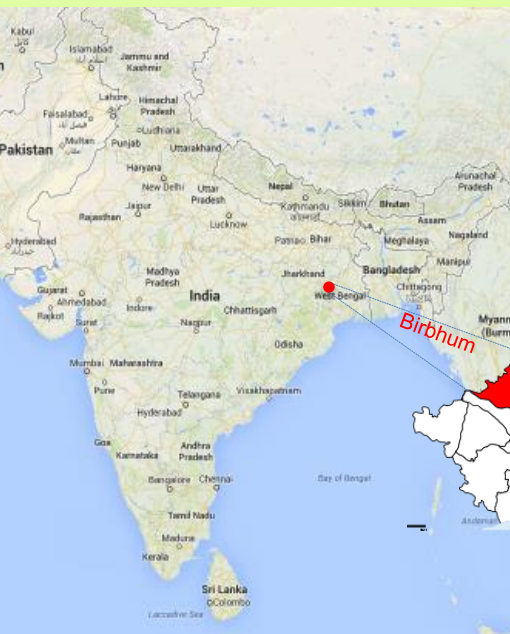
West Bengal

Birbhum district of West Bengal which is typical of rural and agricultural setting.
Birbhum Population Project , a health and demographic surveillance system.

Anthropometric measures and metadata of the subjects

| Anthropo-metric measures	Category	Values	Overall nutritional status
Z-score 1	Height for age	-0.63 to -4.16	
Z-score 2	Weight for age	-1.07 to -4.32	
Z-score 3	Weight for height	-1.6 to - -3.10	
Cumulative Z-score – Cumulative nutritional Index		-11.58 to -2.18	**Apparently healthy** > -6 **Borderline malnourished** -9 < to < -6 **Severely malnourished** < -9

Based on the cumulative nutritional index, the 20 gut metagenomes were divided into three groups as AH, BM and SM

Variation of microbial groups with NS
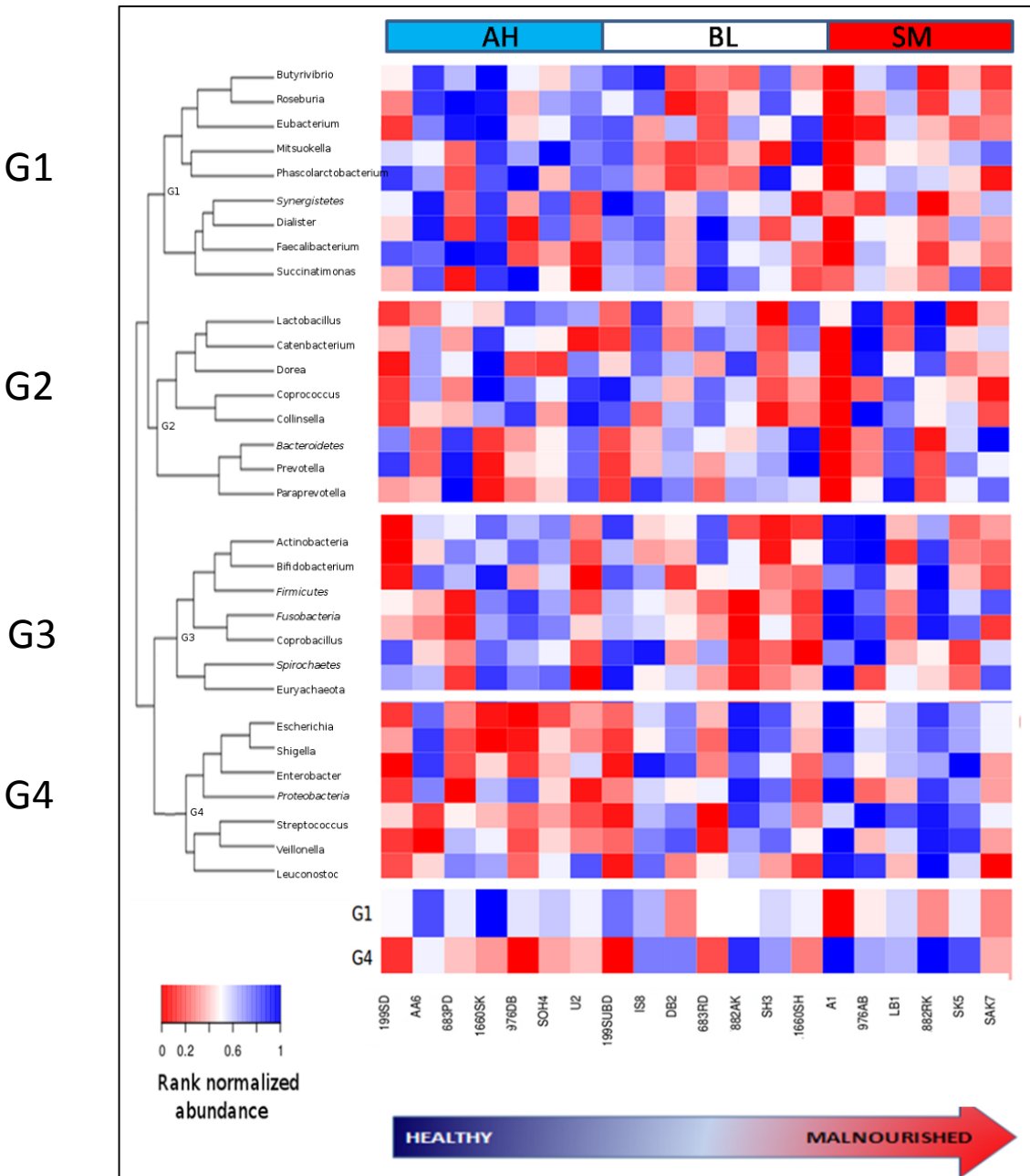
□ **Metagenomes are arranged in the bottom panel (from left to right) in decreasing order of their cumulative nutritional scores**

□ **Taxa are arranged vertically on the left panel as a hierarchical tree based on the similarities in their abundance patterns**
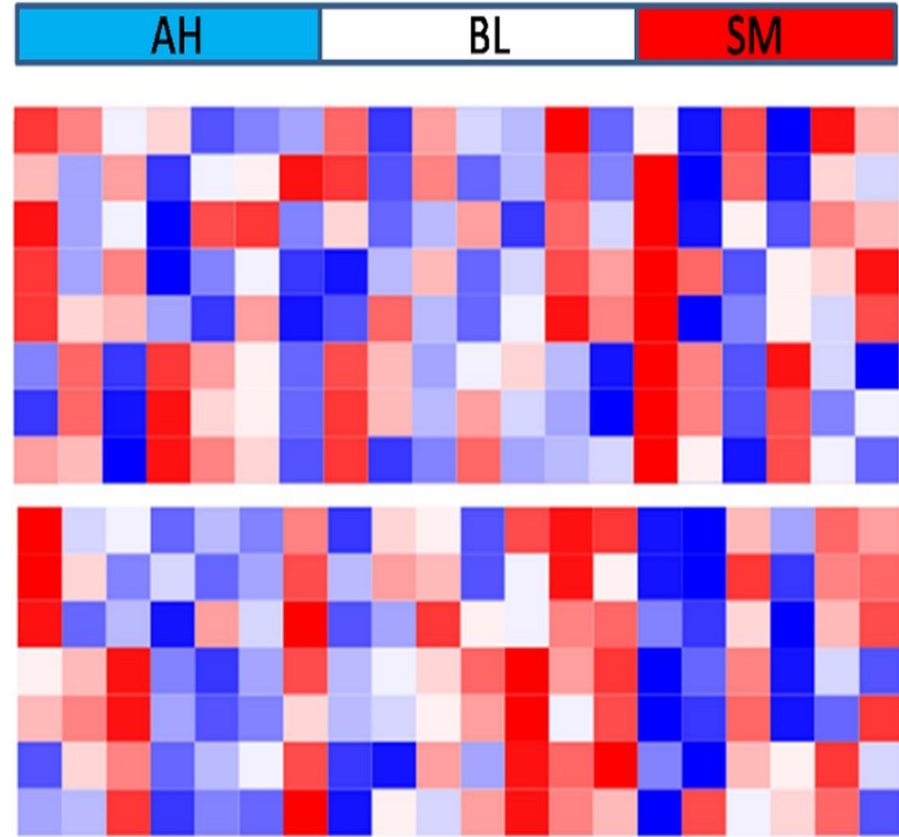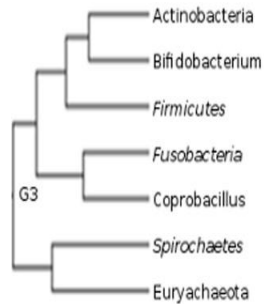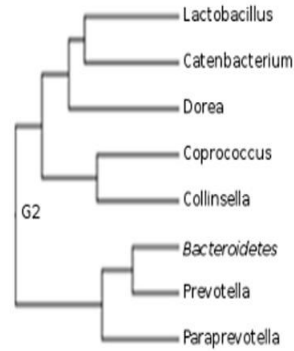
Group 1 and Group 4
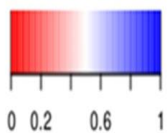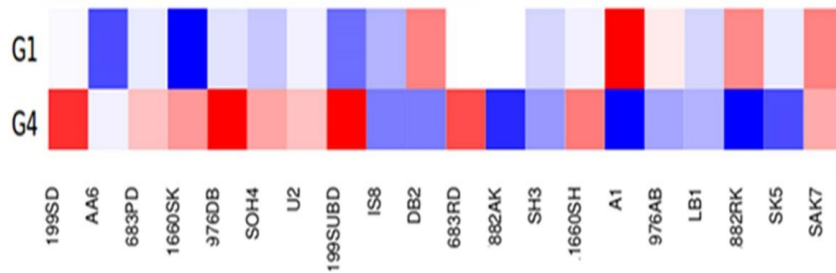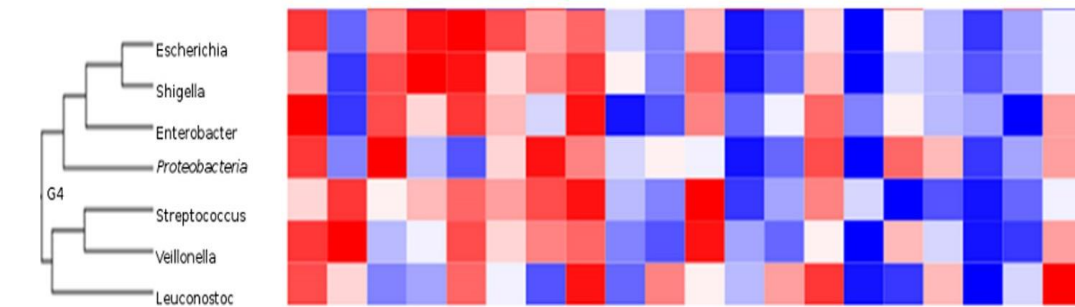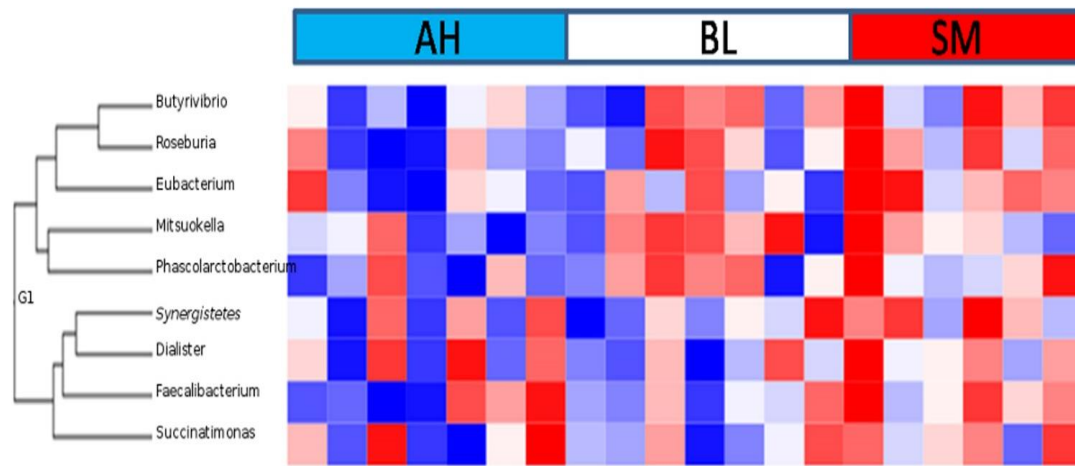
❑ **Taxa belonging to G1 showed progressive decrease in their rank normalized abundances with decreasing nutritional status. Several genera belonging to this group namely Roseburia, Faecalibacterium, Butyrvibrio, had significant positive correlation with nutritional index**

❑ **Taxa belonging to G4 were observed to have a progressive increase in their abundances with the decreasing nutritional index.**
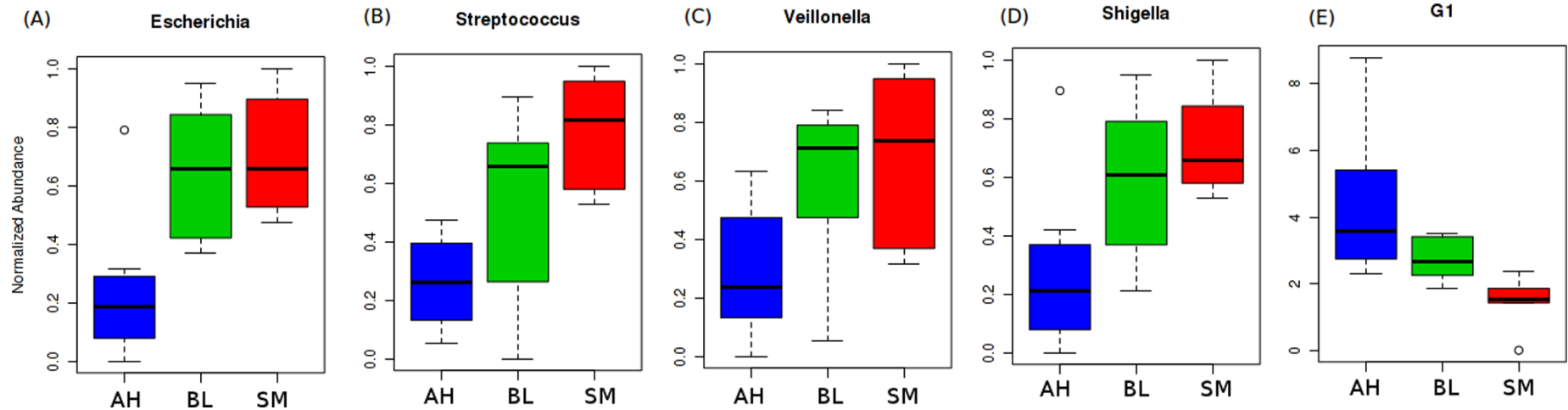
Groups 2 and G3

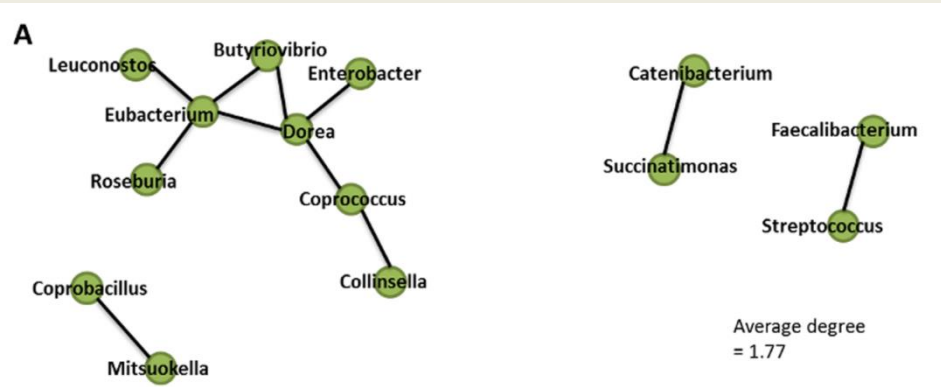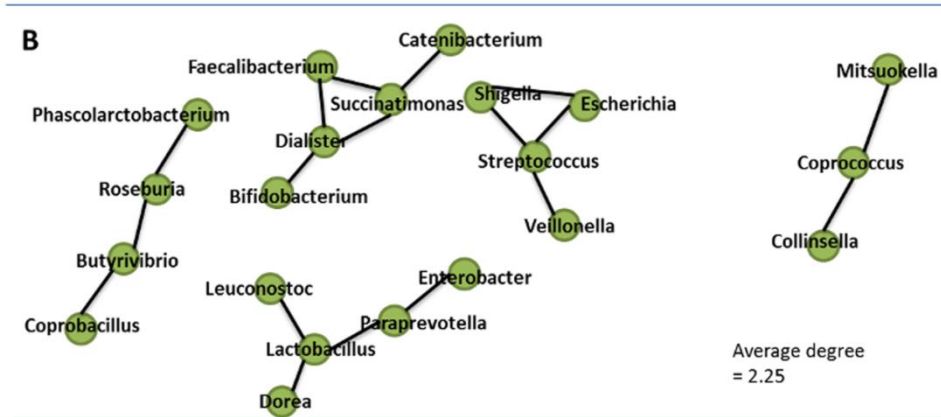# Box-plots showing the abundances of the taxonomic groups significantly differing across the three nutritional categories
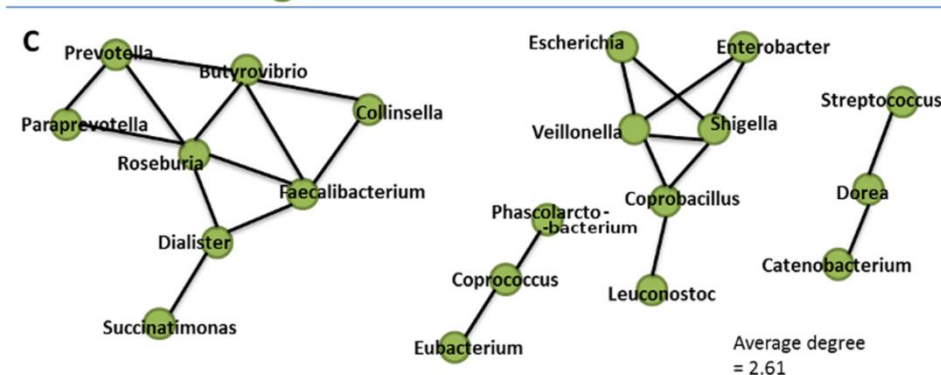
Network of co-occurring genera for the three nutritional status

- ❑ **Despite having contrasting trends in abundance, some of them showed strong positive associations amongst each other**
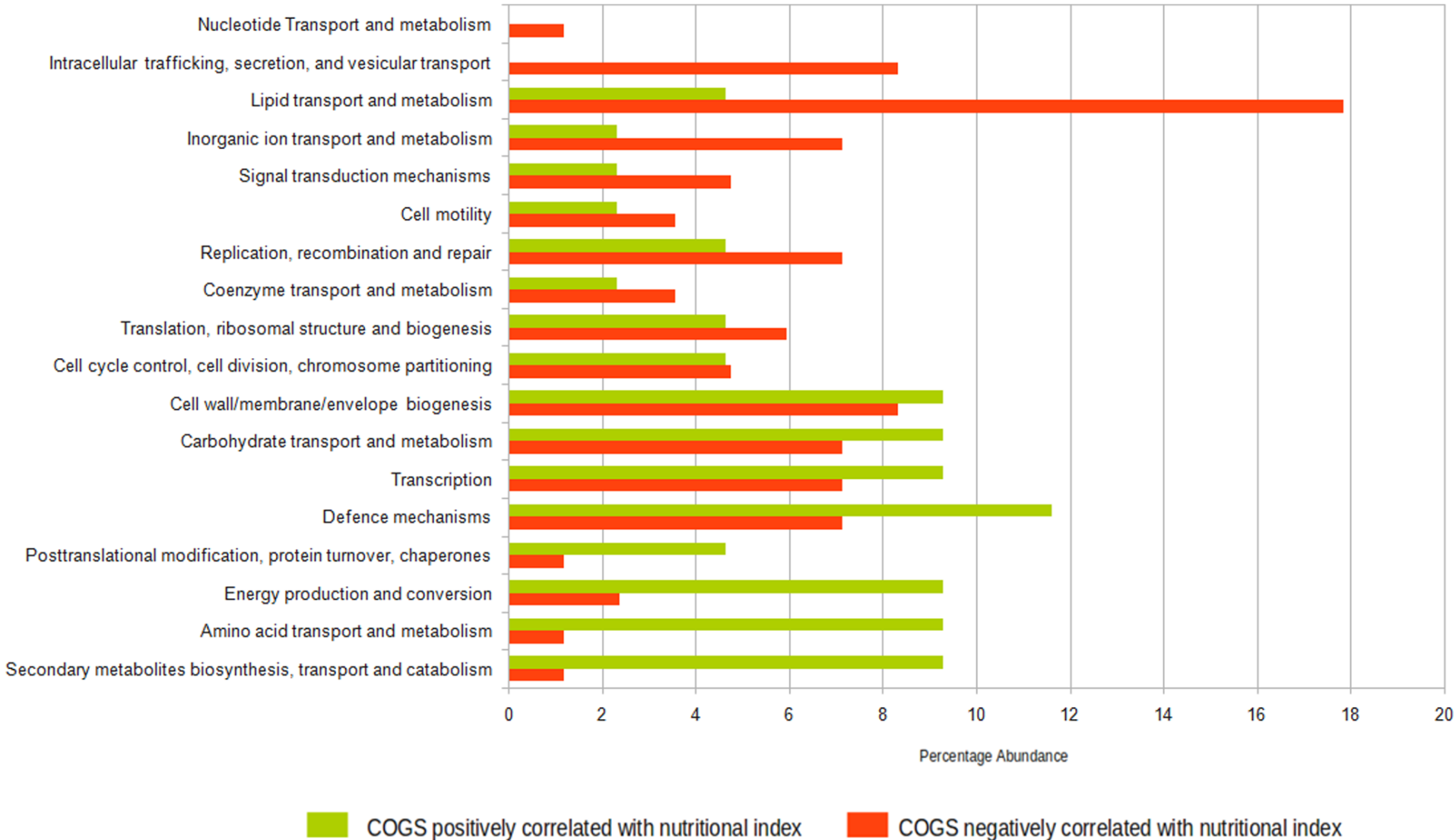
- ❑ **With decrease in NS the pathogenic genera come together in a single connected hub**

- ❑ **The increasing interdependence among the genera is even more pronounced for the SM group of metagenomes**

Profile of the relative abundances of the different functional categories

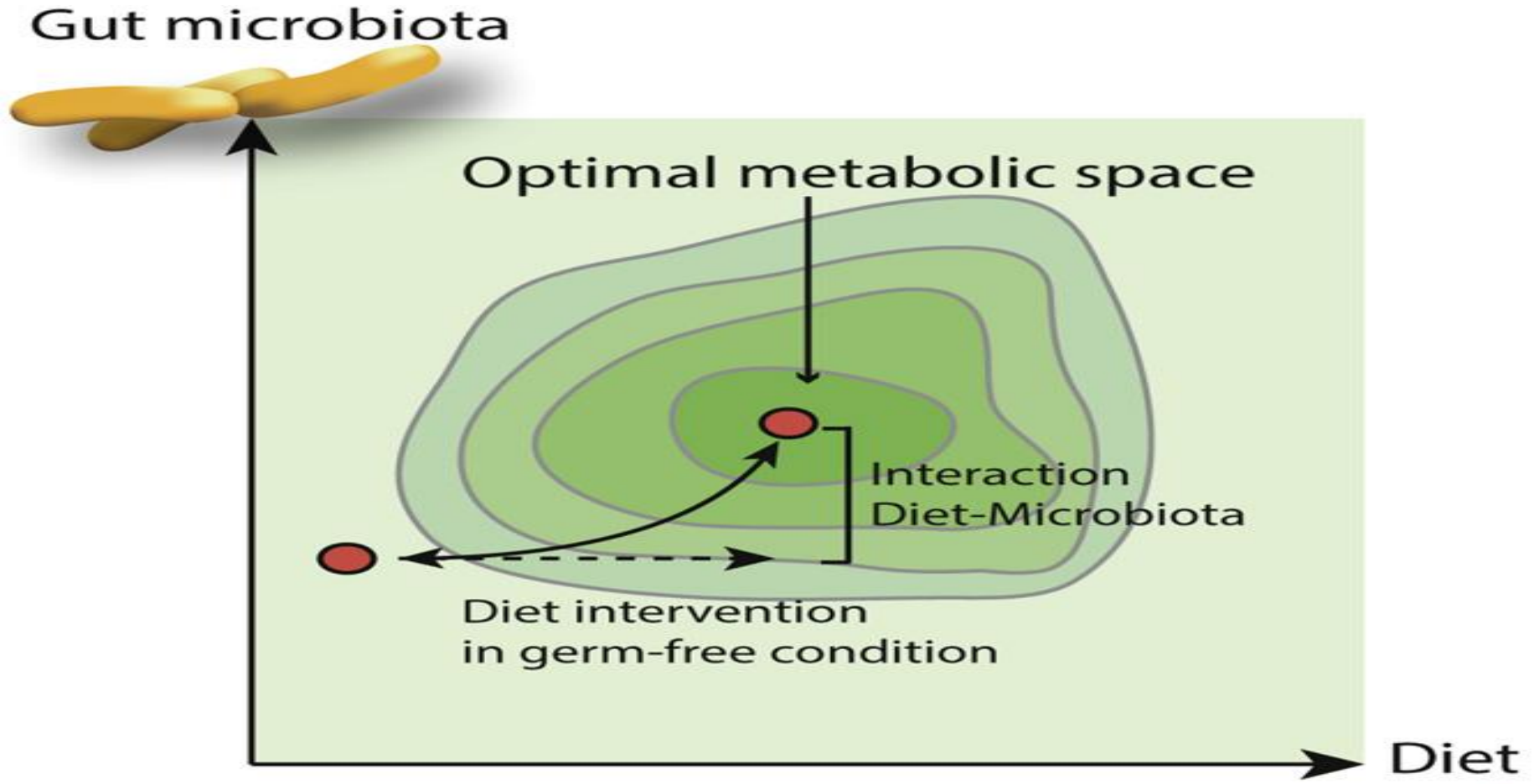# Abundance patterns of CAZyme families across gut metagenomes with varying nutritional status
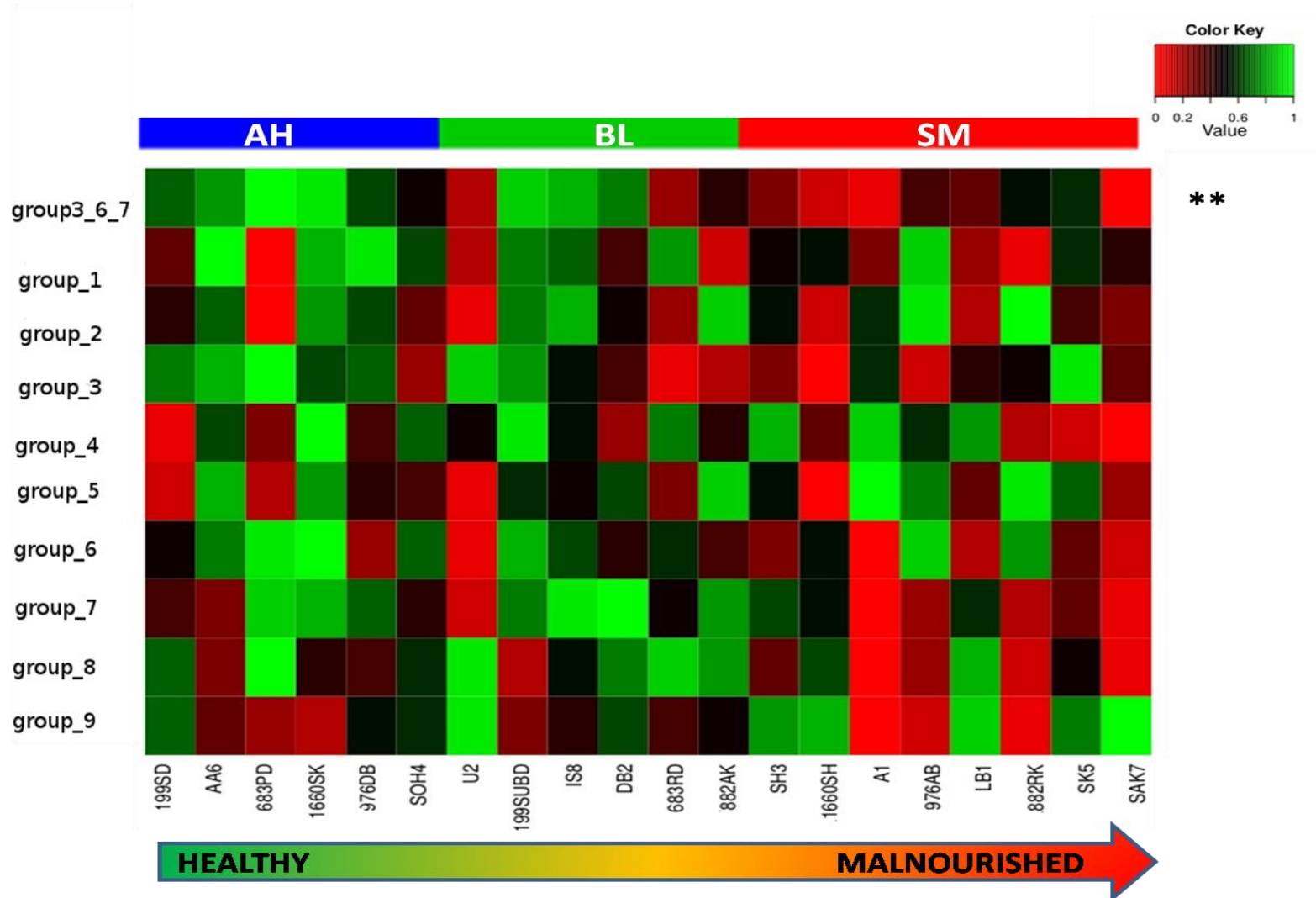
Gr 3 and Gr 6 CAZyme families are known to degrade complex plant carbohydrates while Gr 7 are mostly peptidoglycan degraders

Key findings in the Indian study

❑ Identified patterns where microbial composition, microbial networks, functional categories, carbohydrate digesting enzymes and virulence factors varies in response to nutritional status.

❑ Impaired nutritional status is not only due to the abundances of likely pathogenic microbial groups but also a depletion of several commensal genera.

❑ Biological basis of such patterns may be inferred but the universality and the biological mechanisms governing the occurrence of these patterns remains to be experimentally verified

Interaction between gut microbiota and diet in the context of undernutrition

Gut microbiota

Optimal metabolic space

Interaction Diet-Microbiota

Diet intervention in germ-free condition

Diet

S.P.Claus. 2013. Cell

Key Messages

- ❑ A clear link between the gut microbiota and SAM.
- ❑ Undernutrition is associated with lower diversity of the gut microbiota.
- ❑ SAM was associated with a more prolonged delay in gut microbiota maturation that was only partially corrected by food interventions.
- ❑ The gut microbiota under nutritional deprivation conditions appears to develop towards a "disease-promoting microbiota."
- ❑ The promotion of a beneficial microbiota by certain diets resulted in marked changes in the microbiota, with prominent increase in certain species.
- ❑ Antibiotic therapy might modify the microbiota and affect the potential for energy extraction.

The Bottom Line

For the treatment of under nutrition to be effective and the response to feeding optimal, well designed functional studies are required for formulating a microbial basis of therapy for severe acute malnutrition

Acknowledgements

Sharmila S. Mande

Tarini S. Ghosh

Abhijit Chowdhury

Neerja Hajela

Bhabatosh Das

Sourav Sen Gupta