



## Comparative Analysis of Gut Bacterial Abundance and Dietary Habits of Two Ethnic Tribes of West Bengal, India - A Pilot Study

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### ABSTRACT

*Ethnic communities and their importance have been documented extensively over the years towards preserving biodiversity, the continuation of ethnomedicinal practices and the holistic understanding of the man-environment conflict. The tribal communities of West Bengal are generously distributed in the major geographically distinct regions due to which we can easily distinguish between their habitats. Along with their habitat specific distinctive features, most of these tribes still prefer a non-urban lifestyle both in the context of their livelihood and the procurement and maintenance of their diet. Here, we report the gut bacterial assemblages and their relationship with the dietary habits of nomadic Sabar tribes of Purulia, Jhargram and their adjacent villages along with the foothill Mech tribes of Mongolian descent of North Kamakhyaguri in West Bengal. Anthropometric measures such as BMI was evaluated to understand the nutritional status of the subjects. In order to evaluate the gut bacterial assembly, 16S rRNA from first fecal matter was subjected to Illumina Miseq™ sequencing. Quality control (FASTQC), taxonomic (QIIME2) and functional (iVikodak) profiling was performed for metagenomic analysis. From our study, Holdemanellella, Catenibacterium and Bacteroides exhibited highest abundances of 11.03%, 20.50% and 35.91% across the Sabar male, female and kid respectively while Prevotella, Bifidobacterium and Bacteroides exhibited highest abundances of 40.51%, 17.8% and 29.96% across the Mech male, female and kid respectively. This study will enable us to evaluate the interplay between dietary practice and indigenous lifestyle, gut bacteriome structure and function in the Sabar and Mech tribes of West Bengal.*

**Keywords:** Pristine Gut, Sabar, Mech, Metagenomics, Gut Bacteriome, Diet.

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### INTRODUCTION

Human gastrointestinal (GI) tract comprises of a diverse bacterial population which contributes towards the overall functioning of host metabolism [1]. The composition of the gut microbiota enables the host to have an adaptable ecosystem, which helps in minimizing the effects of dietary and cultural variations. An adequate layout of the core bacteriome of a specific population can lead to the understanding of the essential bacterial functioning [2]. Colonization of the gut by microbes depends on a few factors, including the age, gender, diet, genetic composition, geographic location and health status of an individual [8]. As the gut microbiota is more strongly correlated with food choices than the classic nutritional account usually applied to nutrition studies, changing one's diet can have a major impact on it [9]. It can be pivotal to understand the importance of diet at the outset of evaluating nutritional status in determining the health status of an individual. Populations maintaining traditional livelihood have increased heterogeneity and divergent bacteriome composition as juxtaposed to the urban gut population [4]. The tribal people, in most cases, are not exposed to the daily dose of over-the-counter (OTC) medications which are readily available in the urban areas. Thus, tribal dietary practice and lifestyle are important factors to consider while

exploring the effect of environment on gut bacterial profiles (GBP) and individual state of health [5]. Among 38 different tribal groups in West Bengal, nomadic Sabars are the tenth-largest tribal group with their habitats in the districts of Purulia, Jhargram, Medinipur, Bankura and their adjacent villages [10]. Historically, they have been inhabitants of the forests, but due to deforestation, environmental degradation, and the implementation of the Forest Protection Act, they are being displaced and relocated to areas near the jungles [11]. The Mongolians evolved their distinctive culture and dietary pattern over centuries, which in turn made the Mongol Empire one of the most contiguous empires in history [14]. However, little is known about the composition of the gut microbiota of tribes of Mongolian descent. Our data enlightens on the above, in context to the gut bacterial abundance from the foothill Mech tribes of Mongolian descent of North Kamakhyaguri in West Bengal. The tribe has relied on natural produce from nearby forests, which would aid to preserve the gut's pristine state far away from processed foods. Besides farming, they have also shown a significant interest towards small game hunting as part of alternative livelihood and socio-religious practice [15]. In this work we investigate and compare the gut bacterial profiles of two separate Sabar and Mech families, each consisting of an adult male and female along with their male kid who is yet to attain puberty, using metagenomic and anthropometric approaches and relate them with their dietary habits and lifestyle.

## MATERIAL AND METHODS

### Data and Sample Collection

This study was conducted on the previously studied Sabar family from Laljol-Pataghor village of Jhargram district, West Bengal, India [16] and the foothill Mech tribe from West Bengal, India. The study centered on an adult male (age: 30 years), female (age: 26 years) and their male child (age: 5 years) who was yet to attain puberty.

The local administration and the respondents were made aware of the necessary approvals and informed consent before the initiation of our study. Collection was performed through two steps:

- a) **Counselling along with Medical Assessment:** The subjects were initially advised regarding the need for the first fecal matter and permitted to consume their regular diet as well as an average of 12 hours of sleep per day. During the sampling tenure, they were found to be healthy and had not suffered from any chronic illnesses or morbid conditions in the previous six months. All anthropometric measurements were taken by using calibrated instruments and following the standard protocol [17]. Measurements like height [(HT) (cm)] was measured by Martin's Anthropometer rod, weight [(WT) (kg)] by digital weighing machine (Omron HN 289). Body Mass Index (BMI) was calculated following standard formulae:  $BMI (kg/m^2) = Weight (kg) / Height (m^2)$  and WHO Asia Pacific cut off [18] was used to assess nutritional status.
- b) **Collection of the first fecal matter:** At 5.30 A.M in the morning, the first fecal matter was collected in sterile containers. Following the process, nutritional intake of the previous 24 hours for all participants were recorded following 24-hour dietary recall method and through Food Frequency Questionnaire (FFQ) [19]. The fecal matter was collected in RNALater™ (Qiagen Inc.), which is an RNA stabilization reagent, as per manufacturer's protocol and packed in sterile containers along and sealed with Parafilm and duct tape for transportation to the sequencing laboratory within 10 hours from the collection time.

### Sequencing and Bioinformatic Analyses

Next-generation sequencing was carried out by using the Illumina Miseq™ platform and the bioinformatic analysis was carried out in accordance with our previously reported protocol [20-23]. Quality control of raw reads and identification of prevalent taxa based on the OTU clustering were performed. SILVA v138 [24], Greengenes [25] and RDP [26] Databases were used for taxonomic identification via QIIME2 [27]. Venn analysis of obtained genera was done by using the web-tool Venny v2.1.0 [28]. Differentially enriched metabolic pathways were elucidated from the 16S rRNA gene data in iVikodak webserver [30].

## RESULTS AND DISCUSSION

### Anthropometric Data

The height and weight measurements of the tribal subjects and calculated BMI is summarized in Table 1 with comparative graph shown as Figure 1. The data contributed to the fact that the male and female individuals of Sabar tribe and the Mech male came under the category of Chronic Energy Deficiency which is an indicator of malnutrition and aligns with their current socio-economic status and lifestyle [33].

### Taxonomic Classification

The quality processed paired end reads (quality parameters shown in Supplementary file) were analyzed with QIIME2 pipeline. We inspected the obtained bacterial abundances to assess specific gut-associated bacterial distribution in the individuals under study (Fig 2). *Holdemanella*, *Catenibacterium* and *Bacteroides* exhibited highest abundances of 11.03%, 20.50% and 35.91% across the Sabar male, female and kid

respectively with *Prevotella*, *Bifidobacterium* and *Bacteroides* exhibiting the same with 40.51%, 17.8% and 29.96% across the Mech male, female and kid (Fig. 2). Furthermore, we analyzed the common and unique gut bacteriome patterns by comparing the bacterial profiles of the Sabar (Fig. 3, *top left*) and the Mech families (Fig. 3, *top right*), which revealed that 36.2% of the gut bacteriome was common among the Sabar members while 14.4%, 18.3% and 5.8% were unique to the male, female and kid gut respectively. Analogously, 35.3% of the gut bacteriome was common among the Mech members while 4.8%, 30.1% and 5.7% were unique to the male, female and kid gut, respectively. Finally, we also compared the inter-tribal GBP across the tribes which showed ~60% of the gut bacteriome was common between them while 21.7% and 18.4% were unique to respective tribes (Fig. 3, *bottom*). We also notice that the similarity between kid gut bacteriome and mother gut bacteriome exceeds that between the kid and father's gut bacteriome (7.3% vs 4.1% for Sabar and 16.9% vs 1.3% for Mech). It is to be noted that for the female subjects from both tribes under our analysis, vaginal delivery of kid had taken place, which substantiates the fact that the mother's gut bacteriome exerts more influence over the formation of the kid's gut bacteriome as compared to the father's [34,35,36] and is in line with our previous observations [16].

### **Functional Enrichment Analyses**

Based on the relative bacterial abundance profile, the core functions were predicted and visualized as a heatmap. A large number of core functional pathways were also predicted, which encompasses both homeotic and response pathways. The heatmap of core functions across all six Sabar and Mech subjects is shown in Fig. 5. The Mech male gut exhibited greater number of enriched pathways as compared to any other gut while Sabar male gut showed the least enrichment. The females of both tribes show a balanced profile with both enriched and non-enriched pathways while the kid guts exhibit drastically different profile with the Sabar kid having much more enrichment than the Mech one. These tribal guts are still protected from over-the-counter (OTC) medicines and antibiotic resistance should also be unusual amidst them. Surprisingly, we found enrichment of several antibiotic resistance pathways in these datasets, namely vancomycin and cationic antimicrobial peptide (CAMP) resistances. Vancomycin resistance was prevalent only in Sabar female and kid gut while CAMP resistance was found in Sabar kid and Mech male gut. This can be explained by the Sabar female having suffered from colitis for which she had taken vancomycin as a prescribed drug. On the other hand, presence of both vancomycin and CAMP resistance has been correlated with presence of soft tissue infections by *Staphylococcus* [49] which suggests that the Sabar kid may have suffered from a latent infection. CAMPs are important innate immune compounds that prevent the colonization of pathogens and contribute to infection clearance [50]. The Mech male's high prevalence of CAMP resistance may be attributed to the presence of a higher load of pathogens in his system.

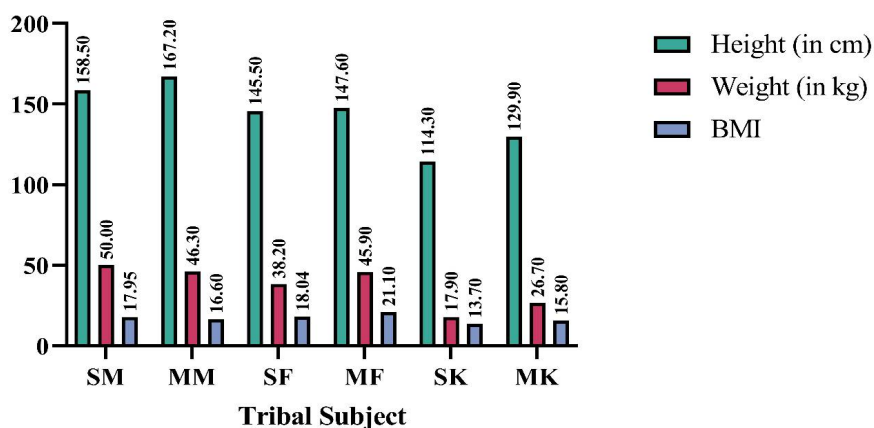
### **Tribal dietary habits and their gut bacterial profiles**

The Sabar community follows a non-vegetarian diet. Rice is their main staple, which they eat twice a day, along with seasonal vegetables such as cabbage, cauliflower, eggplants, tomatoes, pumpkins, radishes, raw papayas, and other wild forest produce. They also include flesh items such as fish, rats, chickens, snails, snakes, which are collected from nearby water bodies or agricultural field. Some individuals in the community have a habit of drinking tea liquor (without milk and sugar) with salt. Additionally, the consumption of alcoholic beverages, both local and traditional, is common among them on a regular basis [31]. Our field studies revealed that these people are still fond of consuming non-timber forest products like roots, tubers, wild fruits, vegetables and wild animals like rats, snakes, civets, rabbits, birds, etc. They have become habituated with everyday staple foods like rice, pulses, vegetables etc., available from the Public Distribution System (PDS) and in the local markets. They used to domesticate cows and buffaloes for obtaining and consuming milk. However, at present, due to the reduction in pastoral area and the high price of those animals, domestication has been vastly reduced. The Mech community's diet consists of a diverse range of locally available vegetables, roots and tubers, pulses, dairy products, and various types of meat. Rice is their staple food, consumed three times a day. Mustard oil is commonly used for cooking purposes. They consume meat from animals such as goats, sheep, buffaloes, fowls, frogs, ducks, as well as eat fish. They have also domesticated animals like buffaloes, goats, and ducks, which provide a regular source of milk, dairy products, and meat. Like many other indigenous communities, consumption of alcoholic beverages also seen among them. They also take tea (without sugar and milk) with salt frequently [32]. The macronutrient intake of the tribal communities and their respective sources have been summarized in Table 2 in supplementary file. Interestingly, on analyzing the relative abundance profile of gut bacteria of each tribal member, we obtained unique abundance profiles of each tribal member even though all of them ingested a more or less similar diet. Across both tribes, we see an abundance of different common gut commensals such as *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Streptococcus* and *Faecalibacterium*, aligning with earlier reports [39]. In addition, for the Sabar male member, we detected a higher percentage of *Succinivibrio*, which can be directly correlated to the glucose fermentation, and since

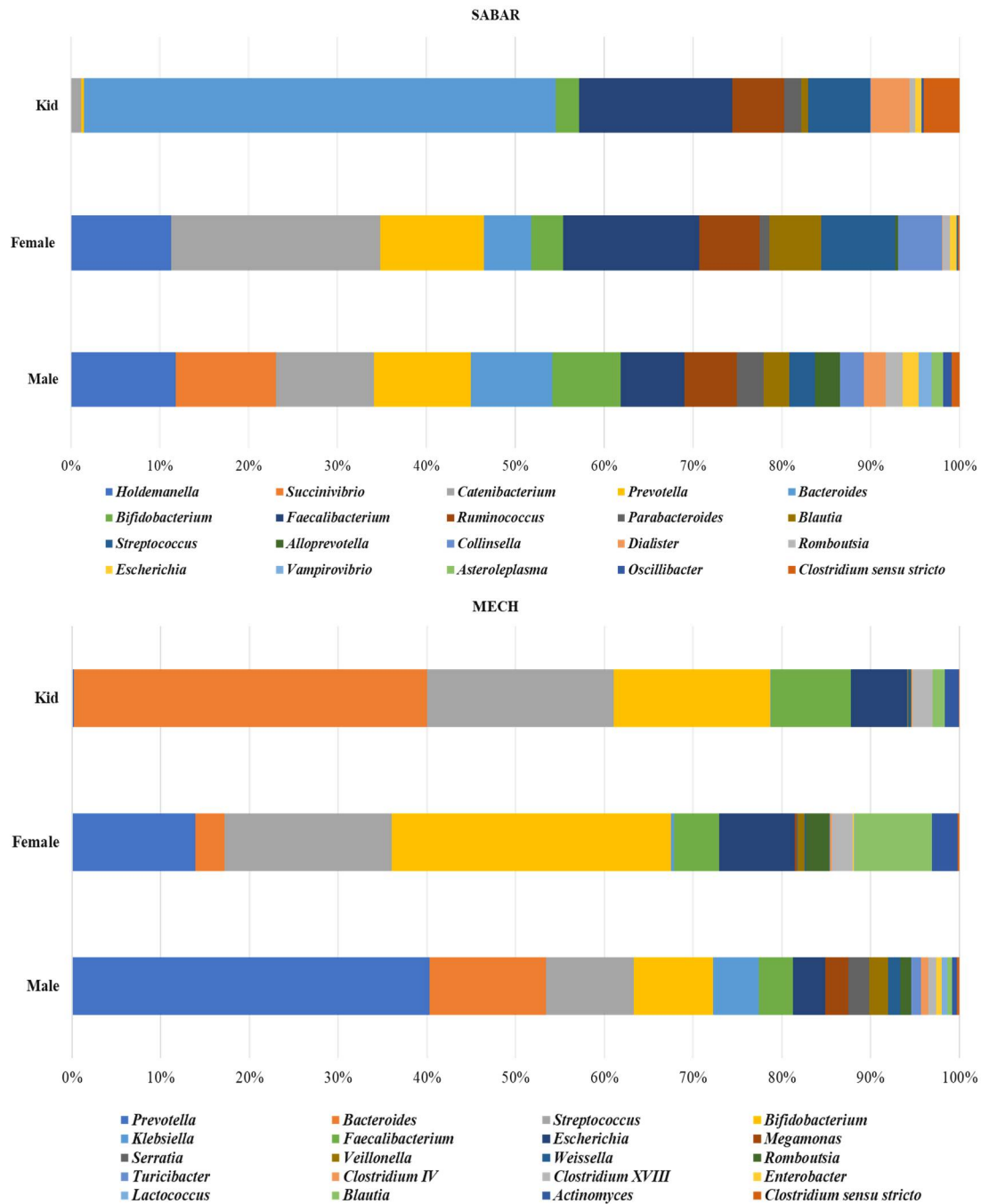
one of the main diet components is Starched Rice, the presence of this bacterial member is readily justified. If we take a closer look into the metabolic pathway enrichment data, we will find that the primary carbohydrate metabolism pathways are enriched in the Sabar male gut profile, which supports the fact that carbohydrate intake is high for the Sabar male under study [31]. As per recent literatures, a vegetarian diet can stimulate the development of *Fecalibacterium*, *Bacteroides*, *Prevotella* and *Clostridium* in the gut [40]. The increasing counts of the above-mentioned gut bacterial species in the gut of Sabar population supports the claim of their consumption of green leafy as well as wild varieties of vegetables in their diet. *Prevotella*, which is an important gut commensal, is found in abundance in both male and female guts of both tribes. Its presence can be attributed to high intake of plant-based carbohydrate [41]. Regular consumption of green leafy vegetables also correlates with increased *Bifidobacterium* abundances across all members under analysis [42]. This observation is similar to reports of similar abundances in the core bacteriome of a few primitive tribes across the world [4,43]. Polyphenols (e.g., from tea) increases abundance of intestinal barrier protectors (*Bifidobacterium* and *Lactobacillus*), butyrate-producing bacteria (*Fecalibacterium* and *Roseburia*), *Bacteroides* and *Akkermansia* but decreases lipopolysaccharide producers (*Escherichia* and *Enterobacter*) [44]. In our study, the Sabar kid displayed an increased *Bacteroides*, *Akkermansia*, *Lactobacillus*, *Faecalibacterium* and *Roseburia* content which can be attributed to his consumption of tea. Similarly, higher abundances of *Bifidobacterium*, *Faecalibacterium* and *Bacteroides* as well as lower abundances of *Escherichia* and *Enterobacter* in Sabar male and female gut can be attributed to their tea consumption. High meat diet correlates with a higher abundance of *Bacteroides* and *Clostridium* and lower counts of *Bifidobacterium* than a meatless diet [41], as is evident from our data which shows higher prevalence of *Bacteroides* in all six gut bacterial profiles. It is interesting to note that both tribal kids have a much higher *Bacteroides* abundance as compared to their parents which may be attributed to the cultural practice in rural households of allocating the bigger chunk of delicacies such as meat to the children in the family. This is the first report of genus *Holdemanella* in the gut bacterial profile of Sabar and Mech tribes. Abundance of this genera has been positively correlated with the consumption of meat such as duck and mutton, dairy products and algae vegetables [45] and high abundance of this genera in Sabar male and female can be justified by their greater consumption of meat products and vegetables. High abundance of this genera in Mech female can be attributed to her consumption of dairy products and duck meat.

**Table 1. Height, Weight and BMI of tribal subjects under analysis. Nutritional status determined based on BMI as per WHO guidelines. SM = Sabar Male, SF = Sabar Female, SK = Sabar Kid, MM = Mech Male, MF = Mech Female and MK = Mech Kid.**

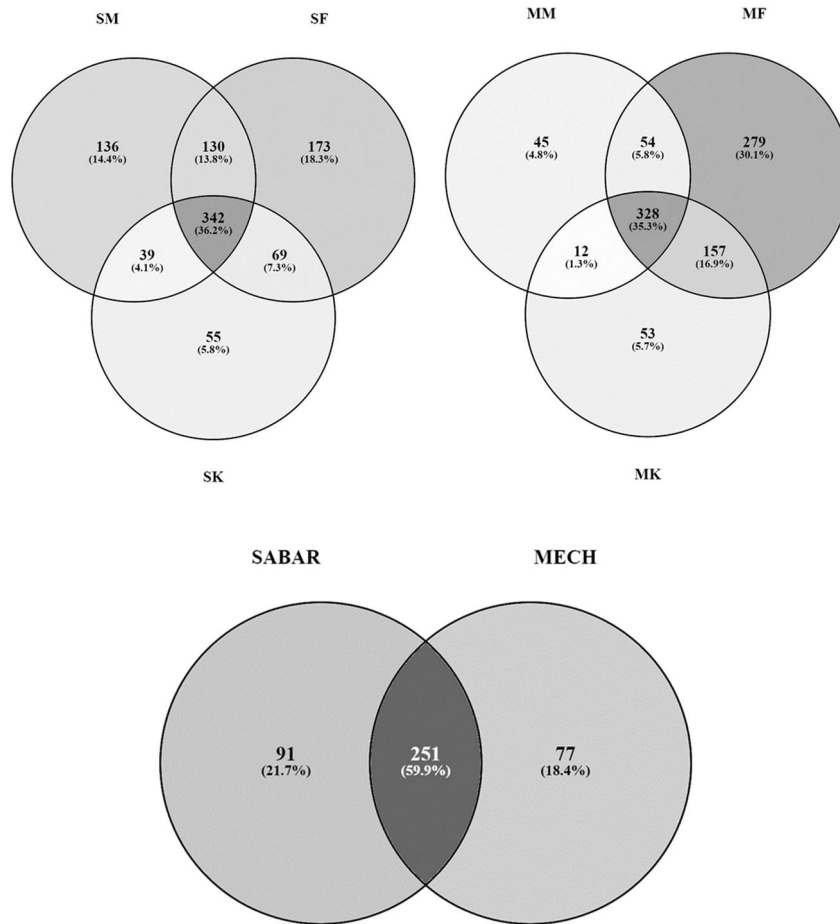
Tribal Subject	Height (in cm)	Weight (in kg)	BMI	Nutritional Category
SM	158.5	50	17.95	Undernutrition
SF	145.5	38.2	18.04	Undernutrition
SK	114.3	17.9	13.7	Normal
MM	167.2	46.3	16.6	Undernutrition
MF	147.6	45.9	21.1	Normal
MK	129.9	26.7	15.8	Normal



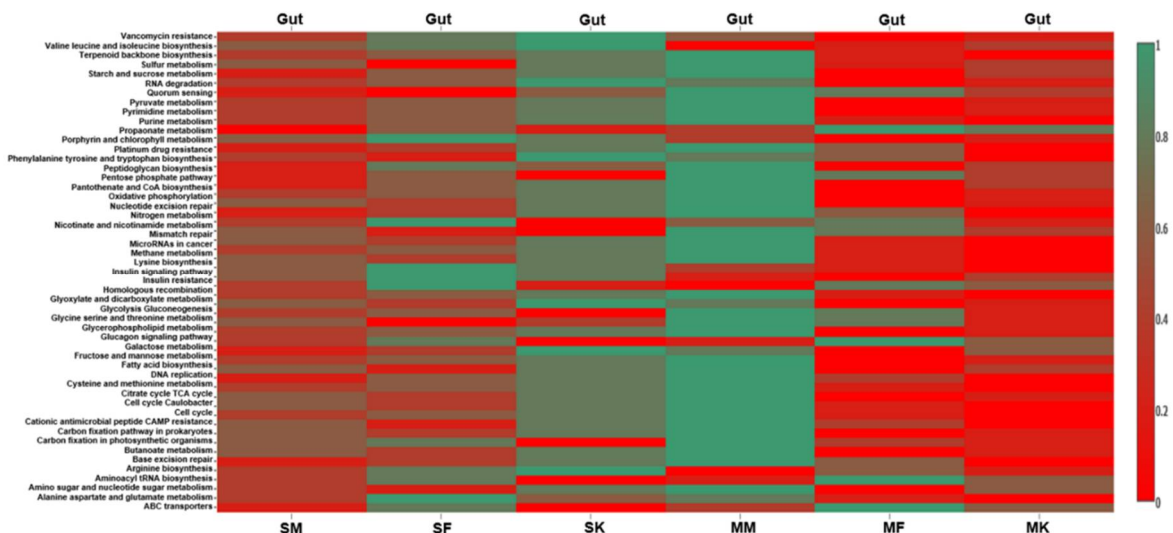
**Figure 1. Comparative visualization of Height, Weight and BMI across the tribal subjects under analysis. SM = Sabar Male, SF = Sabar Female, SK = Sabar Kid, MM = Mech Male, MF = Mech Female and MK = Mech Kid.**



**Figure 2. Comparative Gut Bacterial Profile of Sabar and Mech family under study. Here, SM = Sabar Male, SF = Sabar Female, SK = Sabar Kid, MM = Mech Male, MF = Mech Female and MK = Mech Kid.**



**Figure 3. Venn analysis to segregate between member-specific and tribe-specific taxa where SM = Sabar Male, SF = Sabar Female, SK = Sabar Kid, MM = Mech Male, MF = Mech Female and MK = Mech Kid.**



**Figure 5. Enriched Metabolic pathways between the Sabar and Mech family members under study visualized as a heatmap. Degree of enrichment is shown alongside as a colour gradient ranging from green (100% enrichment) to red (0% enrichment).**

**CONCLUSION**

Few interesting pointers emerge from the study regarding the impact of diet and underlying gut bacteriome of both adult and child in the Sabar and Mech family. It seems that traditional diet practices have resulted in the maintenance of a standard core gut bacterial composition for these members of the Sabar and Mech

tribe represented by in the ~60% commonality of the six gut profiles. Metagenomic predictions based on gut bacterial abundances have predicted the prevalence of several diseases whose propensity to manifest due to genetic susceptibility needs to be validated through SNP mapping studies in the future. In addition, exploration of the gut's microbial profile and its comparison with other tribal communities may help us to comprehend the structural and functional diversity of the microbial consortia at play in the tribal guts. Assessing how the dietary practices can modulate the gut microbiota of the tribes under study will open the doorway towards development of novel dietary therapeutic interventions which may be more acceptable to their indigenous way of life and will have less perturbatory effects on their gut composition, thereby maintaining the pristine state of these guts.

#### DATA AVAILABILITY

Data has been uploaded in NCBI server. The accession numbers are respectively **SRX5459403**, **SRX5459389**, **SRX5459385**, **SRX10752763**, **SRX10759329**, **SRX10766711** for Sabar Kid, Sabar Female, Sabar Male, Mech Kid, Mech Female and Mech Male respectively.

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#### ETHICS STATEMENTS

This research has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Research and Ethics Committee of Bangabasi College, University of Calcutta (No. 002/2017).

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