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COMPARATIVE ANALYSIS OF GUT BACTERIAL ABUNDANCE AND DIETARY HABITS OF TWO ETHNIC COMMUNITIES OF WEST BENGAL, INDIA - A PILOT STUDY

Souradip Basu[1], Gaurab Aditya Dhar[2], Kaustav Das[3], Mahashweta Mitra Ghosh[1], Rajat Banerjee[4],
Subrata Sankar Bagchi[5], and Sayak Ganguli[2*]

[1]Postgraduate and Research Department of Microbiology, [2]Postgraduate and Research Department of Biotechnology, St. Xavier's College (Autonomous), Kolkata - 700 016, West Bengal (India)
[3]Department of Anthropology, Bangabasi College, Kolkata - 700 009, West Bengal (India)
[4]Department of Biotechnology and Dr. B.C. Guha Centre for Genetic Engineering and Biotechnology, University of Calcutta, Kolkata - 700 019, West Bengal (India)
[5]Dr. B.R. Ambedkar Chair, Department of Anthropology, University of Calcutta, Kolkata - 700 019, West Bengal (India)
*****e-mail: *sayakganguli@sxccal.edu*

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ABSTRACT

Tribal communities of West Bengal (India) are generously distributed in the major geographically distinct regions in easily distinguished habitats. Most of these tribes prefer a non-urban lifestyle both in the context of their livelihood and the maintenance of their diet. Here, we report the gut bacterial assemblages along with the dietary habits of Proto-Australoid 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 height, weight and BMI was evaluated to understand the nutritional status of participants. In order to evaluate 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. The study revealed that *Holdemanella*, *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 evaluated the interplay between dietary practice and indigenous life-style, gut bacteriome structure and function in Sabar and Mech tribes.

Keywords: Gut bacteriome, pristine gut, Sabar, Mech, metagenomics

INTRODUCTION

Human gastrointestinal (GI) tract comprises of a diverse bacterial population which contribute to the overall functioning of host metabolism (Finegold *et al.*, 1983). The composition of 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 essential bacterial functioning (Kulkarni *et al.,* 2019). The colonization of gut by microbes depends on factors like age, gender, diet, genetic composition, geographic location and health status of an individual (Senghor *et al.,* 2018). As the gut microbiota is more strongly correlated with food choices than the classic nutritional account usually applied to the nutrition studies, changing one's diet can have a major impact on it (Johnson *et al.*, 2019). It is vital to understand the importance of diet in assessing the nutritional and health status of an individual.

Populations maintaining the traditional livelihood have increased heterogeneity and divergent bacteriome composition as juxtaposed to the urban gut population (Smits *et al.*, 2017). The tribal people, in most cases, are not exposed to over-the-counter 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 the environment on gut bacterial profiles (GBP) and individual state of health (Balgir, 2011).

West Bengal state (India) has a sizeable section of tribal population comprising 5.5% of the state population. Among 38 different tribal groups in West Bengal, Sabars are the tenth-largest tribal group with their habitats in the districts of Purulia, Jhargram, Medinipur, Bankura and their adjacent villages (Gorain *et al.*, 2024). Historically, Sabars have been the inhabitants of forests, but due to deforestation, environmental degradation, and the implementation of Forest Protection Act, they are being displaced and relocated to areas near the forests (Das *et al.*, 2020). Consequently, the majority of Sabars have to rely on agriculture and wage labour as their main sources of livelihood. This transition from their traditional way of life has led to changes in their diet, resulting in a significant reduction in their consumption of various native forest foods. The prevalence of undernutrition has been increasing among adult men and women in the Sabar community, as reported in Orissa, India (Bose *et al.*, 2006), and Purulia, West Bengal (Das *et al.*, 2020).

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 (Zhang *et al.*, 2014). 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 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 shown a significant interest towards small game hunting as part of alternative livelihood and socio-religious practice (Christian, 2018). The present work was aimed to 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.

MATERIALS 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 (Ganguli *et al.*, 2019) and a family from the foothill Mech tribe of West Bengal, India. The study centered on an adult male (age: 32 years), female (age: 28 years) and their male child (age: 7 years) who was yet to attain puberty. The local administration and respondents were made aware of the necessary approvals and informed consent before the initiation of study.

Counselling along with medical assessment

The participants 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 h sleep per day (WHO, 2000). During sampling tenure, they were found healthy and had no history of any chronic illnesses or morbid conditions in the last 6 months. All anthropometric measurements were taken by using calibrated instruments and following the standard protocol (Weiner and Lourie, 1981). The height (cm) was measured by Martin's Anthropometer rod, and weight (kg) by digital weighing machine (Omron HN 289). Body mass index (BMI) was calculated using the standard formulae: BMI (kg m^{-2}) = Weight (kg)/ Height (m^2) and WHO Asia Pacific cut off (WHO, 2000) was used to assess nutritional status.

Analyses of dietary patterns

The initial phase of study involved the collection of first fecal samples at 5:30 am. Thereafter, dietary intake data pertaining to the prior 24 h was ascertained from all participants via a 24-h recall and a Food Frequency Questionnaire (FFQ) [NNMB, 2009]. This guideline provides an idea of the dietary patterns of the participants before sampling. FFQ consisted of few pertaining questions like their traditional food types, eating frequency of the studied subjects under study, food preference avoidance, any allergic reactions to some particular foods or dietary restrictions, meal intake pattern during breakfast, lunch, snacks and dinner [with time and quantity] of the subjects under study, etc. The fecal matter was collected in RNALater™ (Qiagen Inc.), which is an RNA stabilization reagent, as per the manufacturer's protocol. Subsequently, the samples were transferred to sterile containers and secured for transport using Parafilm and duct tape. This ensured sample integrity during delivery to the sequencing laboratory, which occurred within 10 h of collection.

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 (Ganguli *et al.*, 2017; Basu *et al.*, 2022; Dhar *et al.*, 2022). Quality control of raw reads and identification of prevalent taxa based on the OTU clustering were performed. SILVA v138 (Quast *et al.*, 2013), Greengenes (DeSantis *et al.*, 2006) and RDP (Maidak *et al.*, 1996) databases were used for taxonomic identification via QIIME2 (Bolyen *et al.*, 2019). Venn analysis of obtained genera was done by using the web-tool Venny v2.1.0 (Oliveros, 2007). Differentially enriched metabolic pathways were elucidated from the 16S rRNA gene data in iVikodak webserver (Nagpal *et al.*, 2019).

RESULTS AND DISCUSSION

Anthropometric data

The age, height and weight measurements of tribal subjects and their calculated BMI and nutritional category is given in Table 1. The quality control parameters are given in Table 2. The data contributed to the fact that the male and female individuals of Sabar tribe and Mech male fall under the category of chronic energy deficiency which is an indicator of malnutrition and aligns with their current socio-economic status and lifestyle (Vernocchi *et al.*, 2020).

Table 1: The age, height, weight and BMI of tribal subjects

Tribal participants	Age (yr)	Height (cm)	Weight (kg)	BMI	Nutritional category
Sabar male	32	158.5	50.0	17.95	Undernutrition
Sabar female	28	145.5	38.2	18.04	Undernutrition
Sabar kid	7	114.3	17.9	13.70	Normal
Mech male	32	167.2	46.3	16.60	Undernutrition
Mech female	28	147.6	45.9	21.10	Normal
Mech kid	7	129.9	26.7	15.80	Normal

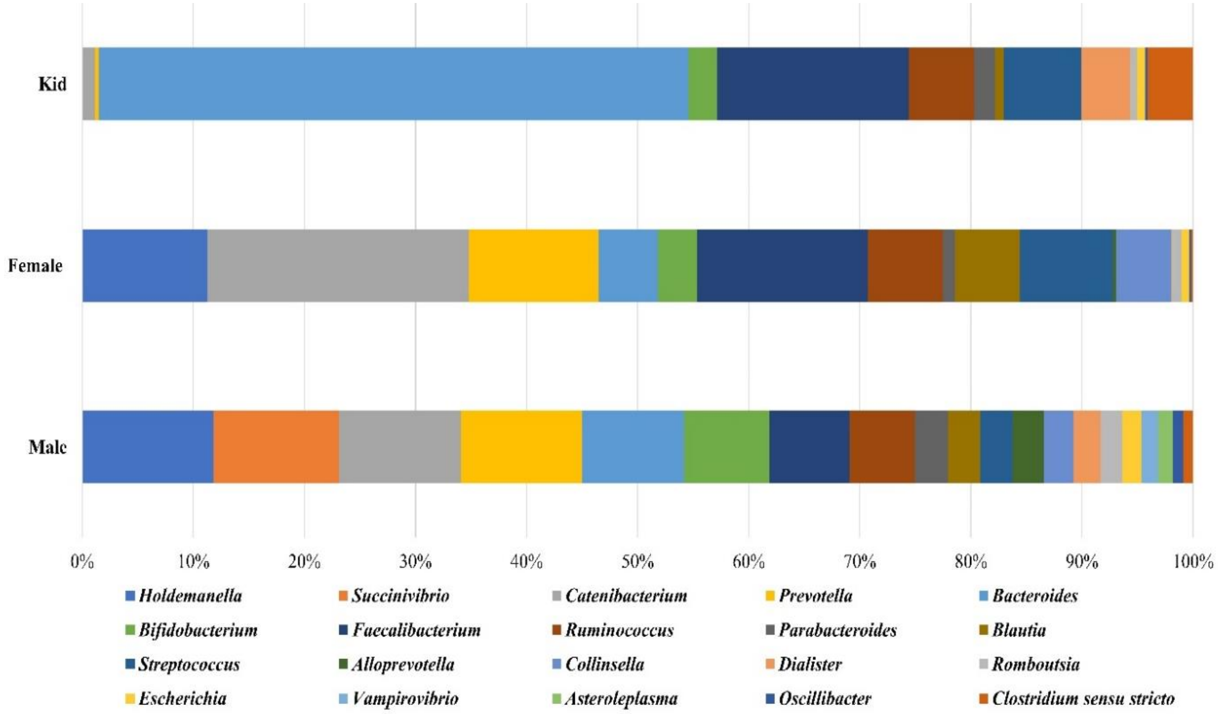
Table 2: QC parameters obtained from FASTQC

Sample name	PE reads (No.)	GC content (%)
Sabar male	1,111,156	54
Sabar female	1,033,126	53
Sabar kid	823,471	54
Mech male	653,562	52
Mech female	1,276,073	55
Mech kid	1,015,190	54

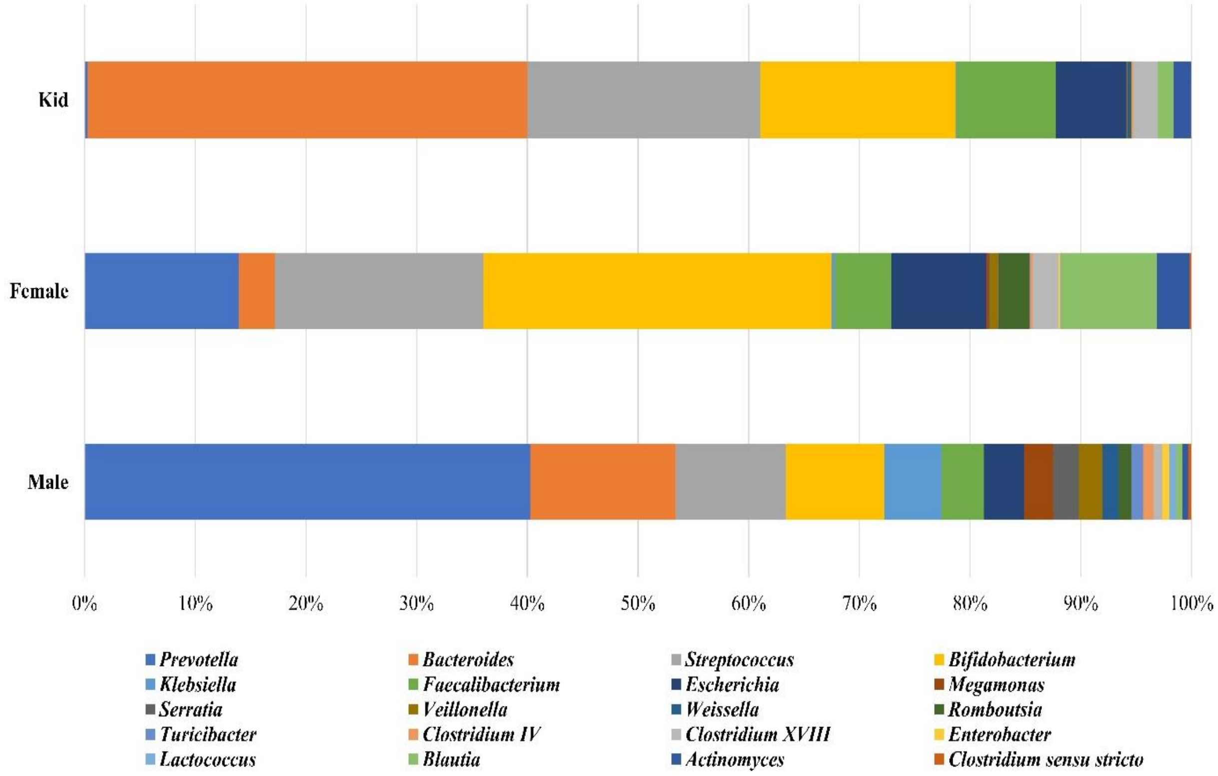
Read length in all was 301 bp; No ambiguous reads were present and the average Phred score was >30 (>Q30) for all the reads.

Taxonomic classification

The quality processed paired-end reads were analyzed with QIIME2 pipeline. We inspected the obtained bacterial abundances to assess specific gut-associated bacterial distribution in the individuals under study (Fig. 1). For Sabar individuals, a total of 1866 OTUs (operational taxonomic units)



A) Sabar family



B) Mech family

Fig. 1: Comparative gut bacterial profile of Sabar (A) and Mech (B) families under study.

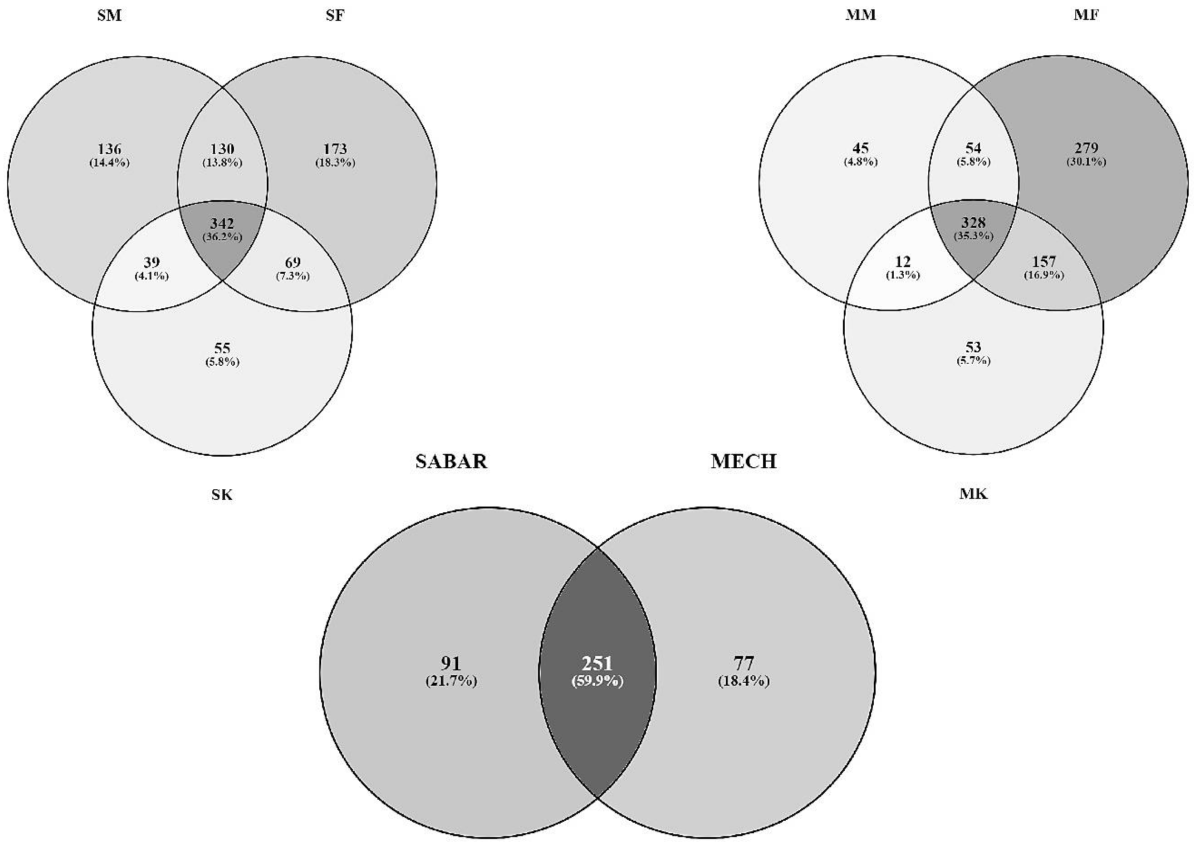


Fig. 2: Venn analysis to segregate between member-specific and tribe-specific taxa. Here**,** SM = Sabar male, SF = Sabar female, SK = Sabar kid, MM = Mech male, MF = Mech female and MK = Mech kid.

were identified with 647, 714 and 505 OTUs observed in male, female and kid through alpha diversity analysis. Of these, *Holdemanella*, *Catenibacterium* and *Bacteroides* exhibited highest abundances of 11.03, 20.50 and 35.91% across the Sabar male, female and kid, respectively. Further, we analyzed the common and unique gut bacteriome patterns by comparing the bacterial profiles of the three Sabar individuals (Fig. 2, top left), which revealed that 36.2% gut bacteriome was common among the three members while 14.4, 18.3 and 5.8% were unique to the male, female and kid gut, respectively.

For Mech participants, metagenomic analysis revealed a total of 1807 OTUs across the three members under study. Alpha diversity analysis revealed 439, 818 and 550 OTUs for male, female, and kid, respectively. From which, *Prevotella*, *Bifidobacterium* and *Bacteroides* exhibited highest highest abundances of 40.51, 17.8 and 29.96% across the Mech male, female and kid, respectively (Fig. 1). Moreover, we compared the GBP of three which revealed that 35.3% gut bacteriome was common among the three members while 4.8, 30.1 and 5.7% were unique to the male, female and kid gut, respectively (Fig. 2, top right). Finally, we compared the inter-tribal GBP across the members of Sabar and Mech families which showed that ~60% gut bacteriome was common, while 21.7 and 18.4% were unique, respectively (Fig. 2, bottom).

Venn analysis revealed a significant similarity between the kid and mother gut bacteriome [7.3% in between Sabar female and kid; 16.9% in between Mech female and kid]; whereas a lesser similarity between kid and father's gut bacteriome [4.1% in between Sabar male and kid; 1.3% in between Mech male and kid] (Fig. 2, top left and top right). It was noted that for female subjects from both tribes under our analysis, vaginal delivery of kid had taken place, which substantiated the fact that the mother's gut bacteriome exerts more influence over the formation of kid's gut bacteriome as compared to the father's (Chu *et al.*, 2017; Wampach *et al.*, 2018; Wang *et al.*, 2020) and is in line with our previous observations (Ganguli *et al.*, 2019).

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 participants is shown in Fig. 3. The Mech male gut exhibited greater number of enriched pathways as compared to any other gut while Sabar male gut showed least enrichment. The females of both tribes showed a balanced profile with both enriched and non-enriched pathways while the kid gut exhibited 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, the presence of both vancomycin and CAMP resistance was correlated with presence of soft tissue infections by *Staphylococcus* which suggested that the Sabar kid may have suffered from a latent infection (Hatlen and Miller, 2021). CAMPs are important innate immune compounds that prevent the colonization of pathogens and contribute to infection clearance (Band and Weiss, 2015). The Mech male's high prevalence of CAMP resistance may be attributed to the presence of a higher load of pathogens in his system.

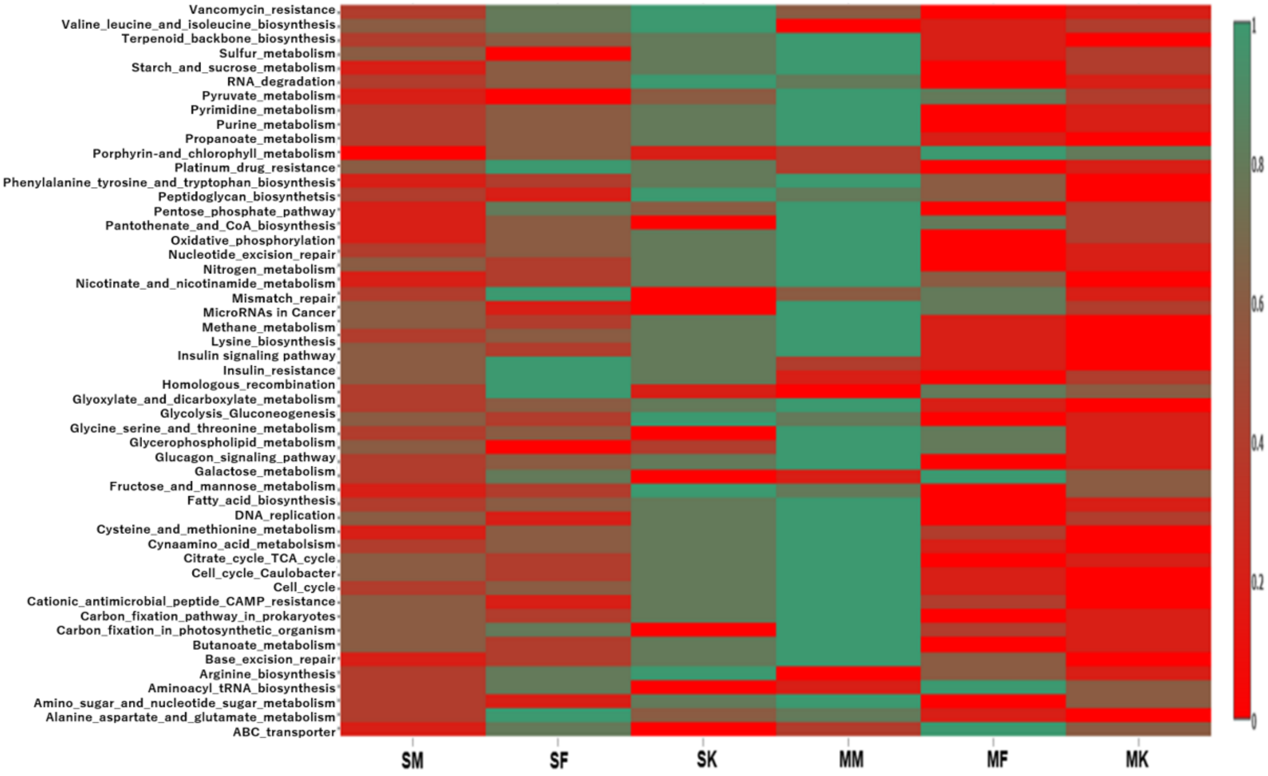


Fig. 3: Enriched metabolic pathways between the Sabar and Mech family members visualized as a heatmap. Degree of enrichment is shown alongside as a colour gradient ranging from green (100% enrichment) to red (0% enrichment). Here, SM = Sabar male, SF = Sabar female, SK = Sabar kid, MM = Mech male, MF = Mech female and MK = Mech kid.

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 fields. 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 (Das *et al.*, 2022). 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. (Table 3). 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. However, at present, due to the reduction in pastoral area and the high price of those animals, domestication has been vastly reduced.

Table 3: Tribal diet profile showing macronutrients and their sources (The Mech kid's diet differed from the parental diet)

Macronutrients	Sabar (Male, female and kid)	Mech (Male & female)	Mech (Kid)
Carbohydrates	Starched rice, roots and tubers like potato, radish, tapioca, etc., Wild vegetables including *Baula audh*, *Chigo*, *Kham audh*, *Churku audh*, *Baya*, *Kulhu audh*, *Muhar audh* and *Bir kundri;* Country liquors like *Hadia*, *Mahua* and *Keya*	Staple rice; Country liquors like fermented rice liquor and fermented millet liquor; Added sugar; Maize, wheat, wheat flour, jaggery, puffed rice, flattened rice, rotis; Biscuits, potato	Staple rice; Country liquors like fermented rice liquor and fermented millet liquor; Added sugar; Maize, wheat, wheat flour, jaggery, puffed rice, flattened rice, rotis; Biscuits, potato; Instant noodles, fast food (Chowmein), sweet-meat (*Laddoo*)
Proteins	Pulses such as lentils, pigeon pea, Bengal gram and green gram; Green leafy vegetables like leaves of colocasia, drum-stick, cabbage and pumpkin, spinach, water spinach and swamp-weeds; Meats of fowls, fish, pork, rat, snakes, frogs, snails and crabs; Eggs	Meats of hogs (wild pig), fowls, ducks, pigeons, goats, deers, turtles, hares and snails; Lentils; Eggs; Fish; Gram (*Chhola*), green leafy vegetables	Meats of hogs (wild pig), fowls, ducks, pigeons, goats, deers, turtles, hares and snails; Lentils; Eggs; Fish; Gram (*Chhola*), green leafy vegetables
Fats	Mustard oil; Meats of pork and rat; Roots and tubers like potato and tapioca; Wild vegetables like *Baula audh*, *Chigo*, *Kham audh*, *Churku audh*, *Baya*, *Kulhu audh*, *Muhar audh* and *Bir kundri*	Ghee; Buffalo milk; Mustard oil; Occasional fast food	Ghee; Buffalo milk; Mustard oil; Occasional fast food (Chowmein): Sweetmeats (*Laddoo*)

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 other indigenous communities, addiction to the consumption of alcoholic beverages is prevalent among them. They also take tea (without sugar and milk) with salt frequently (Sanyal, 1973). Interestingly, on analyzing the relative abundance profile of gut bacteria of each tribal member, we obtained unique abundance profiles from the participants even though all of them ingested a more or less similar diet. Across both tribes, abundance of different common gut commensals such as *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Streptococcus* and *Faecalibacterium* was observed which aligns with earlier reports (Dehingia *et al.*, 2015). In addition, for Sabar male, 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. Closer look at metabolic pathway enrichment data revealed that the primary carbohydrate metabolism pathways are enriched in Sabar male gut profile. This supports the fact that carbohydrate intake is high in Sabar male under study (Das *et al.*, 2022).

As per recent literature, a vegetarian diet can stimulate the development of *Faecalibacterium*, *Bacteroides*, *Prevotella* and *Clostridium* in the gut (Riaz *et al.*, 2017). 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 the high intake of plant-based carbohydrates (Salonen and Vos, 2014). Regular consumption of green leafy vegetables also correlates with increased *Bifidobacterium* abundances across all members under analysis (Seo *et al.*, 2020). This observation is similar to reports of similar abundances in the core bacteriome of a few primitive tribes across the world (Smits *et al.*, 2017; Rinninella *et al.*, 2019).

Polyphenols (e.g., from tea) increase abundance of intestinal barrier protectors (*Bifidobacterium* and *Lactobacillus*), butyrate-producing bacteria (*Faecalibacterium* and *Roseburia*), *Bacteroides* and *Akkermansia* but decreases lipopolysaccharide producers (*Escherichia* and *Enterobacter*) (Moreno-Indias *et al.*, 2016). 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 (Salonen and Vos, 2014), 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 tribe. Abundance of this genera in Sabar male and female can be justified by their greater consumption of meat products and vegetables (Zhang *et al.*, 2022).

Conclusion: Few interesting pointers emerge from this study regarding the impact of diet on the underlying gut bacteriome of both adult and child in Sabar and Mech family. It seems that traditional diet practices have resulted in the maintenance of a standard core gut bacterial composition for the members of Sabar and Mech tribes represented in ~60% commonality of 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 future. In addition, the exploration of gut microbial profile and its comparison with other tribal communities may help us to comprehend the structural and functional diversity of microbial consortia in tribal guts. Assessing how the dietary practices can modulate the gut microbiota of 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 to the NCBI server. The accession numbers are 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 study was conducted in accordance with the Code of Ethics of World Medical Association (Declaration of Helsinki) and was approved by the Research and Ethics Committee of Bangabasi College, University of Calcutta vide No. 002/2017.

Declaration of competing interest: The authors declare no conflict of interest.

Author's contribution: Souradip Basu: Investigation, formal analysis, writing the original draft, visualization, validation; Gaurab Aditya Dhar: Investigation, formal analysis, writing the original draft, visualization; Kaustav Das: Investigation, formal analysis, writing the original draft; Mahashweta Mitra Ghosh: Supervision, resources, review & editing of the original draft; Rajat Banerjee: Supervision, resources; Subrata Sankar Bagchi: Conceptualization, methodology, supervision, review & editing of the original draft, funding acquisition; Sayak Ganguli: Conceptualization, methodology, investigation, supervision, writing the original draft, review & editing, fund acquisition.

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