Intestinal microbiota profile in healthy Saudi children: The bacterial domain

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Abstract

Background: Knowledge of microbiota in health is essential for clinical research on the role of microbiota in disease. We aimed to characterize the intestinal microbiota in healthy Saudi children.

Methods: In this community-based study, stool samples were collected from a randomly selected sample of 20 healthy school children of Saudi origin. The samples were frozen at –80°C till analysis. Bacterial DNA was isolated and libraries were prepared using the Illumina Nextera XT library preparation kit. Unassembled sequencing reads were directly analyzed and quantified for each organism’s relative abundance. The abundance for each organism was calculated and expressed as the average relative percentage from phyla to species.

Results: The median age was 11.3 (range 6.8-15.4) years, and 35% of them were males. The three most abundant phyla were Firmicutes, Bacteroidetes, and Actinobacteria accounting for 49%, 26%, and 24%, respectively. The most abundant genera included Bifidobacterium, Bacteroides, and Blautia accounting for 18.9%, 12.8%, and 8.2%, respectively. Finally, the most abundant species included 14 species belonging to the genus Bacteroides and nine species belonging to Bifidobacterium.

Conclusions: The abundance of intestinal microbiome in healthy Saudi children is different from that of other populations. Further studies are needed to understand the causes of variation between populations, which might lead to new preventive methods and treatment strategies of diseases caused by microbial dysbiosis.

Keywords: Bacteriome, children, gut microbiome, Saudi Arabia

INTRODUCTION

It has been estimated that the gut, particularly the colon, harbors most of the total body microbiota.¹ Although the microbiome of healthy individuals is relatively stable by the age of 3 years, it is modulated throughout the entire lifespan by different environmental factors such as dietary lifestyle, antibiotic treatment, and stress. It has been demonstrated that microbiota is essential to the development and maturation of the immune system. For example, Bacteroides fragilis stimulates T-cell–dependent immune responses important for the development and homeostasis of the immune system.²⁻⁴ Similarly, Lactobacillus...
and *Bifidobacterium* exert a barrier effect to protect the host against pathogens.\[5-7\] Other functions of microbiota, most commonly Clostridia species such as *Ruminococcus* and *Faecalibacterium*, involve the production of short-chain fatty acids (SCFAs) from the digestion of starches and dietary fibers, mainly represented by acetate, propionate, and butyrate. SCFAs have been shown to alter chemotaxis and phagocytosis, induce reactive oxygen species, change cell proliferation and function, have antimicrobial effects, and alter gut integrity. These findings highlight the role of SCFAs as a major player in maintenance of gut and immune homeostasis.\[8\] Other beneficial effects of SCFA include provision of energy and production of vitamins.\[9\]

The microbiome composition is influenced by genetics, mode of delivery at birth, geographic environment, antibiotics, and dietary lifestyle.\[10-13\] Most of the literature on intestinal microbiota are from socioeconomically developed populations and there is a need for studies from other populations which have different genetics and lifestyle. Therefore, we aim to characterize the microbiome profile in a cohort of healthy children in the Kingdom of Saudi Arabia (KSA).

**SUBJECTS AND METHODS**

**The study population**

The children were enrolled from King Fahad Medical City Children Hospital, Ministry of Health, in Riyadh, KSA. Stool samples were collected from 20 healthy school children taken from a large random sample of controls recruited for a mass screening study.\[14\] All children were on a normal family diet and were drinking from the same water sources (bottled and desalinated) at the time of sample collection. In addition, all children had no history of antibiotic intake for at least 6 months prior to sample collection.

**Sample Collection, Storage, and Retrieval**

Stool samples were collected in cryovials and stored at −80°C at the central laboratory in the College of Medicine, King Saud University. At the time of analysis, the samples were retrieved and dispatched by express mail in a temperature-controlled container filled with dry ice until delivery to the laboratory for metagenomic, bioinformatic, and statistical analyses (CosmosID Inc., Rockville, MD, USA).

**DNA Isolation and Sequencing**

DNA was isolated from the stool samples using the DNeasy PowerSoil DNA kit (Qiagen, Hilden, Germany), with each process done according to the manufacturer’s instructions. Isolated DNA was quantified by Qubit (Thermo Fisher Scientific, Waltham, MA, USA).

**Bioinformatic and Abundance Analysis**

Unassembled sequencing reads were directly analyzed with the CosmosID bioinformatics platform (CosmosID Inc.), as described elsewhere for microbiome analysis and quantification of each organism’s relative abundance.\[15-18\] Briefly, the system uses curated genome databases and a high-performance data-mining algorithm that rapidly disambiguates hundreds of millions of metagenomic sequence reads into the discrete microorganisms engendering the sequences. The abundance of each organism was calculated and expressed as the average relative percentage from phyla to species.

**Ethical Approval**

This study was approved by the Institutional Review Board of the College of Medicine, King Saud University, Riyadh, KSA (no. 14/4464/IRB). All children and their parents were informed, and one of the parents signed written consent for the children to participate in the study.

**RESULTS**

**The Study Population**

The study population included 20 Saudi children. The median age was 11.3 (range 6.8-15.4) years, and 35% of them were males. The Saudi family food consumption consists of daily consumption of rice (92%), bread (32%), red meat (45%), chicken (45%), and fish (5%), with a good to poor participation of children in family meals as reported by the mothers (unpublished data). In addition to family food, the dietary lifestyle of the children in this study included daily or twice-weekly consumption of fast food in 7/20 (35%) and 10/20 (50%), respectively, sweet soft drinks in 11/20 (55%) and 4/20 (20%), respectively, fruit in 1/20 (5%) and 7/20 (35%), respectively, vegetables in 9/20 (45%) and 6/20 (30%), respectively, and milk or milk products in 16/20 (80%) and 3/20 (15%), respectively. Finally, 16/19 (84%) of the children received breast milk in the first 2 years of life, with a median duration of 2 months (unpublished data).

**The Abundance of Microbiota**

The average abundance of bacterial microbiota from...
The fecal microbiota profile from phyla to family level is presented in Table 1. The three most abundant phyla were Firmicutes, Bacteroidetes, and Actinobacteria, accounting for an average abundance of 49%, 26%, and 23%, respectively, whereas Proteobacteria were rare (1%). At the class level, the three most abundant organisms were Clostridia (Firmicutes phylum), Bacteroidia (Bacteroidetes phylum), and Actinobacteria (Actinobacteria phylum), accounting for 42%, 26%, and 19%, respectively, whereas at the order level, Clostridiales (Clostridia class), Bacteroidales (Bacteroidia class), and Bifidobacteriales (Actinobacteria class) accounted for 42%, 26%, and 19%, respectively, and at the family level, Lachnospiraceae (Lachnospirales order-Clostridia class), Bacteroidaceae (Bacteroidales class), and Ruminococcaceae (Clostridiales order) were the three most abundant organisms in 24%, 13%, and 12%, respectively.

The average abundance of the top 50 genera is presented in Table 2 with Bifidobacterium, Bacteroides, and Blautia representing 18.9%, 12.8%, and 8.2%, respectively. Finally, the average abundance of the top 100 species shown in Table 3 was dominated by 14 species belonging to the genus Bacteroides and nine species belonging to the genus Bifidobacterium. Lactobacillus and Prevotella, although less abundant, have major functions.

**DISCUSSION**

Information on microbiota in health is important for...
studies related to the association of certain microbes with diseases. Dysbiosis is defined as any change in the composition of microbial communities in any condition relative to the community found in healthy individuals.\textsuperscript{[20‑23]} Accordingly, knowledge of microbiota in health is crucial to the definition of disease-associated dysbiosis. Diet is the most important modifiable modulator of the microbiome and in view of the variability of dietary lifestyle among populations, variation in microbiota is expected.\textsuperscript{[20‑28]} Two types of diets have been most associated with alteration of the microbiome. The Mediterranean diet (MD) is generally regarded as a healthy diet. It is characterized by a combination of complex carbohydrates rich in fiber (cereals, vegetables, fruits), polyunsaturated fatty acids with antiatherogenic and anti-inflammatory items (olive oil, nuts), and bioactive compounds with antioxidative properties, such as flavonoids, phytosterols, terpenes, and polyphenols.\textsuperscript{[20‑24]} In addition, abundant micronutrients

### Table 3: Abundance of the top 100 bacterial species

| No. | Organism | Abundance | No. | Organism | Abundance |
|-----|----------|-----------|-----|----------|-----------|
| 1   | Actinomyces sp. ICM47 | 0.0003 | 26  | Bifidobacterium catenulatum | 0.027 |
| 2   | Akkermansia muciniphila | 0.006 | 27  | Bifidobacterium kashinohense | 0.015 |
| 3   | Alistipes ihumii | 0.006 | 28  | Bifidobacterium longum | 0.021 |
| 4   | Alistipes onderdonkii | 0.008 | 29  | Bifidobacterium mericicum | 0.001 |
| 5   | Alistipes putredinis | 0.026 | 30  | Bifidobacterium pseudocatenulatum | 0.02 |
| 6   | Alistipes shahii | 0.008 | 31  | Blautia obeum | 0.013 |
| 7   | Anaerostipes hadrus | 0.013 | 32  | Blautia sp. KLE 1732 | 0.02 |
| 8   | Bacteroides caccae | 0.005 | 33  | Blautia wexlerae | 0.03 |
| 9   | Bacteroides clarus | 0.002 | 34  | Clostridium sp. L2‑50 | 0.02 |
| 10  | Bacteroides dorei | 0.01 | 35  | Clostridiales bacterium | 0.02 |
| 11  | Bacteroides faecis | 0.003 | 36  | Christensenella minuta | 0.002 |
| 12  | Bacteroides fragilis | 0.011 | 37  | Christensenella tenisonis | 0.002 |
| 13  | Bacteroides intestinalis | 0.003 | 38  | Clostridiales bacterium VE202‑14 | 0.005 |
| 14  | Bacteroides ovatus | 0.01 | 39  | Clostridium saudense | 0.0004 |
| 15  | Bacteroides sp. 3_1_40 A | 0.006 | 40  | Clostridoides difficile | 0.003 |
| 16  | Bacteroides sp. 4_3_47 FAA | 0.003 | 41  | Clostridium sp. L2‑50 | 0.003 |
| 17  | Bacteroides sp. D20 | 0.003 | 42  | Clostridium sp. SS2/1 | 0.007 |
| 18  | Bacteroides uniformis | 0.027 | 43  | Collinsella aerofaciens | 0.011 |
| 19  | Bacteroides vulgatus | 0.014 | 44  | Collinsella sp. 4_B_47 FAA | 0.011 |
| 20  | Bacteroides massiliensis | 0.001 | 45  | Coprococcus catus | 0.005 |
| 21  | Bacteroides pyogenes | 0.0002 | 46  | Coprococcus comes | 0.01 |
| 22  | Barnesiella intestinohominis | 0.005 | 47  | Coprococcus eutactus | 0.0034 |
| 23  | Bifidobacterium adolescentis | 0.051 | 48  | Coprococcus sp. ART55/1 | 0.007 |
| 24  | Bifidobacterium angulatum | 0.021 | 49  | Desulfovibrio piger | 0.01 |
| 25  | Bifidobacterium animalis | 0.002 | 50  | Dialister invisus | 0.01 |
| 51  | Dialister succiniphilus | 0.011 | 76  | Parabacteroides sp. 20_3 | 0.001 |
| 52  | Dorea formicigenerans | 0.01 | 77  | Parabacteroides sp. D13 | 0.004 |
| 53  | Dorea longicatena | 0.021 | 78  | Paraprevotella clara | 0.001 |
| 54  | Dorea sp. AGR215 | 0.004 | 79  | Phascolarctobacterium sp. CAG: 207 | 0.001 |
| 55  | Eggerthella sp. HGA1 | 0.004 | 80  | Prevotella copri | 0.031 |
| 56  | Erysipelotrichaceae bacterium 21_3 | 0.001 | 81  | Prevotella stercorea | 0.001 |
| 57  | Erysipelotrichaceae bacterium 6_1_45 | 0.001 | 82  | Roseburia hominis | 0.005 |
| 58  | Escherichia coli | 0.003 | 83  | Roseburia intestinalis | 0.003 |
| 59  | Eubacterium ramulus | 0.004 | 84  | Roseburia inulinivorans | 0.01 |
| 60  | Eubacterium ventriosum | 0.001 | 85  | Ruminococcus bicirculans | 0.004 |
| 61  | Faecalibacterium prausnitzii | 0.048 | 86  | Ruminococcus bromii | 0.017 |
| 62  | Gordonibacter pamelaeanae | 0.001 | 87  | Ruminococcus callidus | 0.004 |
| 63  | Holdemanella biformis | 0.004 | 88  | Ruminococcus lactaris | 0.003 |
| 64  | Intestibacter bartlettii | 0.002 | 89  | Ruminococcus sp. 5_1_39 BFAA | 0.027 |
| 65  | Lachnospiraceae bacterium 1_1_57 FAA | 0.002 | 90  | Ruminococcus sp. SR1/5 | 0.008 |
| 66  | Lachnospiraceae bacterium 3_1_46 FAA | 0.001 | 91  | Senegalimassilia anaerobia | 0.003 |
| 67  | Lachnospiraceae bacterium 5_1_63 FAA | 0.012 | 92  | Streptococcus thermophilus | 0.02 |
| 68  | Lachnospiraceae bacterium 8_1_57 FAA | 0.002 | 93  | Subdoligranulum sp. 4_3_54 A2 FAA | 0.005 |
| 69  | Lactobacillus ruminis | 0.002 | 94  | Subdoligranulum variabile | 0.001 |
| 70  | Megasphaera sp. BL7 | 0.001 | 95  | Sutterella wadsworthensis | 0.0002 |
| 71  | Megasphaera elsdenii 1.25\textsuperscript{[a]} | 0.002 | 96  | Tannerella sp. 6_1_58 FAA_CT1 | 0.0001 |
| 72  | Odoribacter sapiensis | 0.002 | 97  | Tyzzerella nexilis | 0.001 |
| 73  | Oscillospiraceae bacterium VE202‑24 | 0.001 | 98  | Veillonella dispar | 0.001 |
| 74  | Parabacteroides distasonis | 0.004 | 99  | Veillonella parvula | 0.001 |
| 75  | Parabacteroides merdae | 0.010 | 100 | Veillonella sp. 6_1_27 | 0.001 |
in this diet including vitamins and minerals help prevent malnutrition and immunodeficiencies. A recent report from a northern Spanish population identified several beneficial bacteria that were more abundant in the individuals with higher adherence to the MD. *Bifidobacterium animalis* was the species with the strongest association with the MD. Some SCFAs-producing bacteria were also associated with MD. The authors concluded that MD, fiber, legumes, vegetable, fruit, and nut intake are associated with an increase in butyrate-producing taxa such as *Roseburia faecis*, *Ruminococcus bromii*, and *Oscillospira (Flavonifractor) plautii*.\[25\] By contrast, Western diet (WD) is considered unhealthy as it is characterized by a high content of unhealthy fats, refined grains, sugar, and reduced content of fruits and vegetables. This leads to changes in gut microbiota and immune system, negatively affecting the gut integrity, and thus promoting local and systemic chronic inflammation.\[26‑30\] Gut microbiota modulated by WD include increased Firmicutes/Bacteroidetes ratio and decreased population of SCFA producers such as *Lachnobacterium* species, leading to intestinal barrier disruption and increased permeability.\[28‑30\] The contrasting effects of MD and WD on gut microbiota suggest variation in gut microbiota between populations, indicating the need for studies from different populations.\[31‑33\]

Gut bacterial microbiota characterized in this study revealed a microbiota profile different from that of other populations. A study comparing gut microbiota in 15 children from rural Burkina Faso (BF) and Florence (Italy) revealed that more than 94.2% of the sequences belonged to the four most common phyla (Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes), which is in a slightly lower abundance than the 99% obtained in our study, but similar to previous reports.\[34\] However, Bacteroidetes was the most abundant phylum (73%), which includes the genus *Prevotella* (53%) and Firmicutes (12%), contrasting with 51% abundance of Firmicutes and only 26% abundance of Bacteroidetes in the European (EU) group. This significant difference in abundance of bacteria between EU and BF samples was attributed to difference in dietary lifestyle. The diet of BF children was low in fat and animal protein and rich in starch, fiber, and plant polysaccharides and was predominantly vegetarian, whereas the diet of EU children was a typical WD high in animal protein, sugar, starch, and fat and low in fiber.\[35\] The profile of microbiota in Saudi children (Firmicutes 49% and Bacteroidetes 26%) was strikingly similar to that of EU children, which is not surprising in view of the similar dietary lifestyle of Saudi children to EU children. Another study comparing fecal microbiota from four healthy 9- to 14-year-old Bangladeshi children with that of four children of the same age range in the USA found important differences. At the phyla level, the abundance of Firmicutes, Bacteroidetes, Tenericutes, Proteobacteria, and Verrucomicrobia was 46%, 43%, 4%, and 2%, respectively, in the US children, contrasting with the abundance of Firmicutes 60%, Bacteroidetes 20%, Tenericutes 12%, and Proteobacteria 5% in Bangladeshi children. At the genus level, *Prevotella*, which belongs to the phylum Bacteroidetes, was the most prevalent genus in Bangladeshi children, while the *Bacteroides* genus, which belong to the same Bacteroidetes phylum, was the most prevalent in the US children.\[36\]

These variations in microbiota profile between populations in Italy, USA, Spain, Bangladesh, and Burkina Faso are most likely related to variations in their dietary lifestyle. The gut microbiome profile of healthy Saudi children in this report is closer to Western than non-Western patterns, an expected finding in view of the similarity of the diet of Saudi children to Western dietary lifestyle.

**CONCLUSION**

To our knowledge, this is the first report on gut microbiota profile in healthy Middle eastern childhood population. Characterization of gut microbiota in this report may serve as controls in dysbiosis research in the KSA and similar countries. However, in the era of probiotic and fecal microbial therapy, there is a need for more studies from other countries, particularly developing countries. Such studies are necessary to understand the causes of variation, which might lead to new preventive and treatment strategies of diseases caused by microbial dysbiosis.

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**Conflicts of interest**

There are no conflicts of interest.

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