Original Article

Intestinal microbiota profile in healthy Saudi children: The bacterial domain

Mohammad El Mouzan, Abdulrahman A. Al-Hussaini[1,2], Ahmed Al Sarkhy, Asaad Assiri, Mona Alasmi

Department of Pediatrics, Gastroenterology Division, King Saud University Medical City, King Saud University, Riyadh, [1]Division of Pediatric Gastroenterology, Children's Specialized Hospital, King Fahad Medical City, Riyadh, [2]Faculty of Medicine, AlFaisal University, Riyadh, Kingdom of Saudi Arabia

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

Address for correspondence: Prof. Mohammad El Mouzan, Department of Pediatrics, Gastroenterology Division, King Saud University, P.O. Box: 2925, Riyadh - 11461, Saudi Arabia.
E-mail: melmouzan@ksu.edu.sa

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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*

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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).

DNA libraries were prepared using the Illumina Nextera XT library preparation kit, according to the manufacturer's protocol. Library quantity and quality were assessed with Qubit and TapeStation (Agilent Technologies, Santa, Clara, CA, USA). Libraries were then sequenced on an HiSeq platform (2 × 150 bp; Illumina, San Diego, CA, 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

Table 1: Fecal microbiota profile from phyla to family level in heathy Saudi children

Level	Organism	Abundance	Level	Organism	Abundance
Phyla	*Actinobacteria*	0.23	Order	*Lactobacillales*	0.03
Phyla	*Bacteroidetes*	0.26	Order	*Veillonellales*	0.02
Phyla	*Firmicutes*	0.49	Order	*Verrucomicrobiales*	0.01
Phyla	*Proteobacteria*	0.01	Family	*Bacteroidaceae*	0.13
Phyla	*Verrucomicrobia*	0.01	Family	*Clostridiaceae*	0.01
Class	*Actinobacteria*	0.19	Family	*Eggerthellaceae*	0.01
Class	*Bacteroidia*	0.26	Family	*Enterobacteriaceae*	0.01
Class	*Clostridia*	0.42	Family	*Erysipelotrichaceae*	0.02
Class	*Coriobacteriia*	0.04	Family	*Eubacteriaceae*	0.02
Class	*Erysipelotrichia*	0.02	Family	*Lachnospiraceae*	0.24
Class	*Gammaproteobacteria*	0.001	Family	*Peptostreptococcaceae*	0.01
Order	*Bacteroidales*	0.26	Family	*Prevotellaceae*	0.04
Order	*Bifidobacteriales*	0.19	Family	*Ruminococcaceae*	0.12
Order	*Clostridiales*	0.42	Family	*Streptococcaceae*	0.02
Order	*Eggerthellales*	0.01	Family	*Tannerellaceae*	0.02
Order	*Erysipelotrichales*	0.02	Family	*Veillonellaceae*	0.02

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

Table 2: Abundance of the top 50 bacterial genera in fecal samples

No.	Organism	Abundance	No.	Organism	Abundance
1	*Actinomyces*	0.002	26	*Intestinimonas*	0.0002
2	*Akkermansia*	0.006	27	*Klebsiella*	0.0004
3	*Alistipes*	0.07	28	*Lachnoclostridium*	0.003
4	*Anaerostipes*	0.013	29	*Lactobacillus*	0.003
5	*Bacteroides*	0.128	30	*Lactococcus*	0.0003
6	*Barnesiella*	0.005	31	*Megasphaera*	0.002
7	*Bifidobacterium*	0.189	32	*Methanobrevibacter*	0.002
8	*Bilophila*	0.001	33	*Odoribacter*	0.003
9	*Blautia*	0.082	34	*Oscillibacter*	0.01
10	*Catenibacterium*	0.004	35	*Oxalobacter*	0.0004
11	*Clostridium*	0.010	36	*Parabacteroides*	0.02
12	*Collinsella*	0.022	37	*Phascolarctobacterium*	0.002
13	*Coprobacter*	0.0002	38	*Porphyromonas*	0.001
14	*Coprococcus*	0.025	39	*Prevotella*	0.033
15	*Desulfovibrio*	0.002	40	*Roseburia*	0.02
16	*Dialister*	0.020	41	*Ruminiclostridium*	0.003
17	*Dorea*	0.032	42	*Ruminococcus*	0.06
18	*Eggerthella*	0.001	43	*Senegalimassilia*	0.003
19	*Enterobacter*	0.001	44	*Streptococcus*	0.023
20	*Erysipelatoclostridium*	0.001	45	*Subdoligranulum*	0.006
21	*Escherichia*	0.003	46	*Sutterella*	0.0003
22	*Eubacterium*	0.022	47	*Tannerella*	0.0002
23	*Faecalibacterium*	0.048	48	*Tyzzerella*	0.001
24	*Holdemanella*	0.004	49	*Veillonella*	0.001
25	*Intestinibacter*	0.002	50	*Weissella*	0.0001

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 kashiwanohense*	0.015
3	*Alistipes ihumii*	0.006	28	*Bifidobacterium longum*	0.021
4	*Alistipes onderdonkii*	0.008	29	*Bifidobacterium merycicum*	0.001
5	*Alistipes putredinis*	0.026	30	*Bifidobacterium pseudocatenulatum*	0.02
6	*Alistipes shahii*	0.008	31	*Bifidobacterium sp.* 12_1_47 BFAA	0.02
7	*Anaerostipes hadrus*	0.013	32	*Blautia obeum*	0.013
8	*Bacteroides caccae*	0.005	33	*Blautia sp.* KLE 1732	0.02
9	*Bacteroides clarus*	0.002	34	*Blautia wexlerae*	0.03
10	*Bacteroides dorei*	0.01	35	*Catenibacterium mitsuokai*	0.004
11	*Bacteroides faecis*	0.003	36	*Christensenella minuta*	0.002
12	*Bacteroides fragilis*	0.011	37	*Christensenella timonensis*	0.002
13	*Bacteroides intestinalis*	0.003	38	*Clostridiales bacterium* VE202-14	0.005
14	*Bacteroides ovatus*	0.01	39	*Clostridium saudiense*	0.0004
15	*Bacteroides sp.* 3_1_40 A	0.006	40	*Clostridioides 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_8_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 intestinihominis*	0.005	47	*Coprococcus eutactus*	0.0034
23	*Bifidobacterium adolescentis*	0.051	48	*Coprococcus sp.* ART55/1	0.007
24	*Bifidobacterium angulatum*	0.01	49	*Desulfovibrio piger*	0.001
25	*Bifidobacterium animalis*	0.02	50	*Dialister invisus*	0.01
No.	**Organism**	**Abundance**	**No.**	**Organism**	**Abundance**
51	*Dialister succinatiphilus*	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. AGR2135*	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 pamelaeae*	0.001	87	*Ruminococcus callidus*	0.004
63	*Holdemanella biformis*	0.004	88	*Ruminococcus lactaris*	0.003
64	*Intestinibacter 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*	0.020
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[05]	0.002	96	*Tannerella* sp. 6_1_58 FAA_CT1	0.0001
72	*Odoribacter splanchnicus*	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

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.[19] 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.[20-23] 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.[20-24] In addition, abundant micronutrients

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.[26,27] 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 Bacteriodetes 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%, 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 research 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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