Extended Abstract Journal of Molecular Microbiology 2021
Vol.5 No.2

Molecular profiling of Gut Microbiota among Egyptian Children and the Impact of Potential Confounding Factors.

Laila Hussein1*, Sa-ngapong Plupjeen2, Massalin Nakphaichit2, Hagar F ElBakry1, SuneeNitisinprasert2

1Department of Nutrition, National research center, Giza, Egypt

2Department of Biotechnology, Faculty of Agro–Industry, Kasetsart University, Chatuchak, Bangkok, Thailand

***Corresponding author:** Laila Hussein, Department of Nutrition, National research centre, Giza, Egypt , E-mail: dr.lailahussein@yahoo.com

1.Abstract

The aim of this study is to establish an age appropriate gut microbiota composition among healthy Egyptian preschool- to school-aged children (3 - 9 years) from the urban Giza governorate. The molecular profiles of 7 predominant gut microbiota constituents were determined by quantitative RT-PCR (qPCR). Group-specific primer pairs targeting the 16S rRNA gene were designed for the identification of the Bacteroides fragilis group, Bifidobacterium spp., Clostridium leptum subgroup, Enterobacteriaceae, Faecalibacterium praunitzii, Lactobacilli spp. and Prevotella group The results showed that the relative abundances of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria averaged 17.3, 23.0, 46.4 and 13.29%, respectively. The ratio of Firmicutes (Sum of Clostridium leptum subgroup +Faecalibacterium praunitzii + Lactobacilli spp)/ Bacteroidetes (Bacteroides fragilis + Prevotella group) averaged 1.52±0.19. The average genome numbers of the four bacterial taxa were significantly higher in boys ($P<0.05$) than in age-matched girls. The mean genome copies of 4 fecal microbiota were higher among children with older siblings in the same household ($P<0.05$) than among unrelated children. Significant familial transmission of Bifidobacterium spp. was found across family brothers and sisters ($0.1<P>0.05$) compared to unrelated children

2. Keywords: Egyptian children - Genomic faecal DNA - Q-RT- PCR- Bifidobacterium spp – Faecalibacterium praunitzii - Firmicutes/Bacteroidetes ratio- Familial siblings

3. Introduction

Neonates from a healthy pregnancy are born sterile [1-3]; by days 4 - 7, the gut is colonized by Enterobacteria and Streptococcus, both of which generate a low redox environment, allowing strict anaerobic bacterial species to flourish [4,5]. Several factors affect this process, such as gestational age6, delivery mode [7,8] breast feeding [9,10] contribution of maternal microbiota and other factors. At 1 week after birth, Bifidobacterium species are present at low concentrations in the faeces, but they become one of the dominant population groups in the infant gut at 3 months of age [11]. The early development of the infant microbiome with non-pathogenic [commensal] microbiota is vital [12] and coincides with critical periods of central nervous system and gut–brain axis development [13]. Breast milk itself contains microbiota, including Bifidobacterium, Streptococcus, and Lactobacillus species [14-16], and its composition is dynamic, changing over time. Currently, discriminative molecular signatures have been provided for distinguishing breastfed [BF] versus formula-fed [FF] infants based on combinations of host gene sets [17]. The faecal microbiota profile of healthy full-term, vaginally delivered, exclusively breast-fed (FTVDBF) infants is currently the standard for a healthy infant microbiota and is a target for improving infant formulas [18]. Bifidobacteria longum is notably abundant in the stool of breast fed infants, representing up to 90% of the total microbiota, and its gene copies in healthy young children were in 3.5-fold excess compared to adults [19,20]. Bifidobacteria longum has enzymes suited to extracting energy from human milk fucosylated oligosaccharides [21] and producing short-chain fatty acids [SCFAs], which lower the pH in the colon, allowing them to outcompete pathogenic bacteria such as E. coli and Clostridium perfringens [22]. During the weaning process and the introduction of complementary and family solid foods, there is major diversification in the composition of the gut microbe community associated with the dominance of Bacteroides [23]. Infants from rural Africa, where the diet is dominated by starch, fibre and plant polysaccharides, had faecal microbiota abundant in the Actinobacteria (10.1%) and Bacteroidetes (57.7%) phyla, while in European children, whose diets are rich in sugar, starch and animal protein, the respective abundance levels of these phyla are reduced to just 6.7 and 22.4% [24].

The intake of high dietary fibre thickens the mucus layers in the colon due to long-term microbial colonization [25], while low dietary fibre is associated with the erosion of the colonic mucus barrier and defects in the intestinal tight-junction proteins [26].The gut microbiota plays a major role in regulating mucosal and systemic immunity particularly secretory IgA (SIgA), which assists in limiting the exposure of the epithelial cell surface to bacteria and mediates the binding of bacterial SIgA receptors [27]. The healthy gut microbiome undergoes a more prolonged development compositional and functional qualities after the first 1 to 3 years of life3 [28]. At weaning and after the introduction of solid foods, the gut are enriched with gene coding allowing the utilization of a larger variety of carbohydrates, vitamin synthesis and xenobiotic degradation [29]. Gut microbiota maturity and microbiota-for-age Z score (MAZ) have been developed, based on a microbial signature of 25 bacterial taxa that were discriminant for age and healthy growth [30]; infants with severe acute malnutrition had a less-mature microbiota than their respective age-matched healthy mates. Another study identified 45 healthy mature anaerobic gut microbiota (HMAGM) by metagenomics and culturomics in Senegalese healthy children, but the faeces of children with kwashiorkor were enriched with the potentially pathogenic Streptococcus gallolyticus [31]. Recently, an ecogroup of 15 covarying bacterial taxa was identified in the guts of 3-to 5-year-old healthy Bangladeshi children; this bacterial taxon could be applicable to healthy children from other low-income countries [32]. Another feature of severe acute malnutrition was the early depletion in gut Bifidobacterium longum, a typical maternal probiotic [33]. The gut microbiota of 7-to 12-year-old children was characterized by its richness with genes supporting development, while the gut communities of adults were enriched with

genes associated with inflammation [34]. There is still no agreement on the age at which the paediatric phylogenetic composition of the bacterial communities reaches the respective adult. According to one study, this varied between the 3- or 4-year period after birth [35], 4- to 8-year-old children [36], the teenage years or even later ages [37]. Dutch children aged between 2 and 18 years showed intraindividual microbial dynamics, particularly for bacteria with enzymes degrading carbohydrates such as Bifidobacterium, Clostridium clostridioforme and Faecalibacterium prausnitzii [38]. Geography- and country-specific microbial signatures have been implicated as one of the major environmental factors in shaping the microbiota composition [39] and characterizing the gut microbiome between different regions within specific populations, such as Europe and Africa24 and the US and Egypt [40]. Normal, healthy gut microbiota is characterized by gut colonization resistance (CR), which disfavours colonization by enteric pathogens [41]. Disturbance of the microbiota and high growth levels of pathogens disrupt colonization resistance (CR). Currently, the appropriate application of omics techniques for identifying microbial ecology supplemented with computer science is able to identify more than>80% of individuals, whereby gene-level codes are more robust to temporal variation than taxon-level codes [42]. There is accumulating evidence on the presence of familial transmission of selected gut microbiota communities among members inhabiting the same household compared to respective patterns among unrelated subjects [43].

The present study aims to establish age – appropriate gut microbiota composition among healthy Egyptian preschool to school aged children (3 - 9 years) from urban Giza governorate and to study the impact of family cohabitation on the transmission of gut microbiota communities.

4. Methods

Study design and Participants

Thirty-five Egyptian boys and girls with a mean age of 7 years (3 – 9 years) were recruited from the kindergartens and primary schools of a single urban Giza governorate. The study protocol received approval from the Medical Research Ethics Committee of the National Research Center. The protocol was fully explained to the mothers of all children, and written informed consent was obtained from them before participation in the trial. All children in the present study were delivered at full term (range, 37 to 42 weeks). Exclusion criteria were defined as any symptoms that were likely related to digestive tract disease and/or intake of antibiotics during the three months prior to the start of the trial. The birth date of each child was recorded from the birth certificate to calculate the age. The anthropometric measurements included weight with light clothing to the nearest 10 grams. Height was measured with a device with a headboard and a fixed measuring tape, marked in millimeters. The numerical values of weight and height were plotted on the appropriate weight and height chart. Growth was evaluated by comparing individual measurements to reference standards, represented by percentile curves on a 2006 WHO growth charts. Data related to socioeconomic characteristics for each child were assessed using a questionnaire sheet and included data related to the parents' education and family size and data related to the hygienic condition of the house. Faecal samples were frozen at −20°C immediately after collection. After frozen transport, the samples were stored at −80°C within 72 h after delivery until DNA extraction.

Faecal DNA Extraction. Faecal specimens (150 mg) were promptly removed from the frozen sample, and complete DNA extraction was performed as described earlier [44] using a ZR faecal DNA isolation kit (DNA Mini Prepkit Catalogue No. D6010, Zymo Research, Ohio, USA) following the manufacturer's protocol. The extraction was completed under simultaneous multidirectional beating in a FastPrep 24 instrument (MP Biomedicals, Ohio, USA) for 40 s. Genomic DNA quantification was based on the absorbance measurement at a wavelength of 260 nm using a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Genomic solution containing 50 µg ml−1 double-stranded DNA had an OD260 of 1.0 and was stored at -70°C. The DNA concentration was adjusted to 10 ng µl-1on the day of amplification.

Target taxon	Primer	Primer sequences	Amplicon size	Annealing T	Reference
Bacteroides	g-Bfra-F	**ATAGCCTTTCGAAAGRAAGAT**	495	53	[92]
	g-Bfra-R	**CCAGTATCAACTGCAATTTTA**			
Bifido	g-Bifid-F	**TCGCGTC(C/T)GGTGTGAAAG**	243	62	[93]
	g-Bifid-R	**CCACATCCAGC(A/G)TCCAC**			
Clostridium	sg-Clept-F	**GCACAAGCAGTGGAGT**	239	55	[92]
	sg-Clept-R3	**CTTCCTCCGTTTTGTCAA**			
Enterobacteriaceae	EN - F	**CATTGACGTTACCCGCAGAAGAAGC**	195	57	[94]
	EN - R	**CTCTACGAGACTCAAGCTTGC**			
Faecalibacteriumpraunitzii	Fpra-F	**CCATGAATTGCCTTCAAAACTGTT**	142	71	[95]
	Fpra-R	**GAGCCTCAGCGTCAGTTGGT**			
Lactobacillus group	Lab-F	**AGCAGTAGGGAATCTTCCA**	341	53	[93]
	Lab-R	**CACCGCTACACATGGAG**			
Prevotella	g-Prevo-F	**CACRGTAAACGATGGATGCC**	513	62	[92]
	g-Prevo-R	**GGTCGGGTTGCAGACC**			

Table 1: 16S rRNA gene-targeting taxon-specific primers sequences their annealing temperature and sizes of PCR product analyzed by quantitative PCR technique

F = Forward primer ; R = Reverse primer

Table 1 lists the sequences of each primer pair targeting 16S rRNA of the following bacterial groups/ species: Bacteroides fragilis group, Bifidobacterium spp., Clostridium leptum subgroup, Enterobacteriaceae, Faecalibacterium praunitzii, and Lactobacillus group and Prevotella spp. The following six pure bacterial groups and species were obtained from the American Tissue culture collection [ATCC] and were used for bacterial identification: Bacteroides fragilis, Bifidobacterium ATCC 15707T, Clostridium leptum, Enterobacteriaceae, Faecalibacterium prausnitzii DSM 17677, Lactobacillus spp and Prevotella nigrescens jcm12250. The bacterial cultures were grown anaerobically in an atmosphere of 80% N2 - 20% CO2 at 37°C. The purity of the cultures was checked by inspecting the colony morphology after anaerobic growth on BHI medium and cellular morphology with Gram staining. The cells were suspended in buffer and centrifuged, and pellets (approximately 109 bacterial cells) were used for the extraction and purification of DNA using an RTP Bacteria DNA Mini Kit (Invitek, Berlin; Germany). The extracted genomic DNA was stored at -70°C until use. Just before measurement, a dilution series from 2.0 x 10 to 2.0×108 colony-forming units/µl PCR was performed. DNA amplification and detection by quantitative polymerase chain reaction were performed using Power SYBR Green PCR Master Mix (Applied Biosystems, USA) in optical-grade 96-well plates in a total volume of 20 µL. The PCR master mix contained the following ingredients: 10 µL of PCR master mix (Applied Biosystems, USA); 0.8 µL each of the appropriate forward and reverse primers; 2µL of faecal genomic DNA template or pure bacterial DNA and 6.4µL DNAse-free water. Melting curves for genomic DNA were generated by a real-time PCR system (Light Cycler 480 Roche) provided with fluorescent lamps at an excitation wavelength of 465 nm and an emission wavelength of 510 nm, and the instructions from the manufacturer were followed. Real-time PCR: Initial heat activation 5 min/95°C, 2-step cycling denaturation 10 s/ 95°C, combined annealing/extension 30 s/60°C and the number of cycles was 35–40. The qPCR standard curves were constructed for each primer pair using the abovementioned serial dilutions of bacterial genomic DNA of known concentration. The qPCR data were converted to the estimate of log10 total genome copies from each bacterial taxon present in one gram faecal wet weight using the appropriate software program.

Data Analyses. Numerical data are presented in Whisker boxplots showing median and interquartile ranges. T-tests were used to compare the distribution of values between the groups. Analysis of variance was carried out to determine the effect of familial cohabitation and the presence of older siblings within the same family on the abundances of each taxon. Differences between mean values were considered significant when $P < 0.05$.

5. Results

The participating children were normal with respect to their growth,

expressed as WAZ with a mean value of 0.72±0.51 (range -1.57 to 3.1) and a mean HAZ value of 0.43±0.49 (range -1.26 to 3.7). Figure (1) illustrates the % relative abundance levels of the four bacterial phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. The phyla Bacteroidetes [B] and Firmicutes [F] were the 2 most dominant phyla in the common core gut, with a mean F/B ratio of 1.52±0.19, and the ratio was not affected by sex or age group.

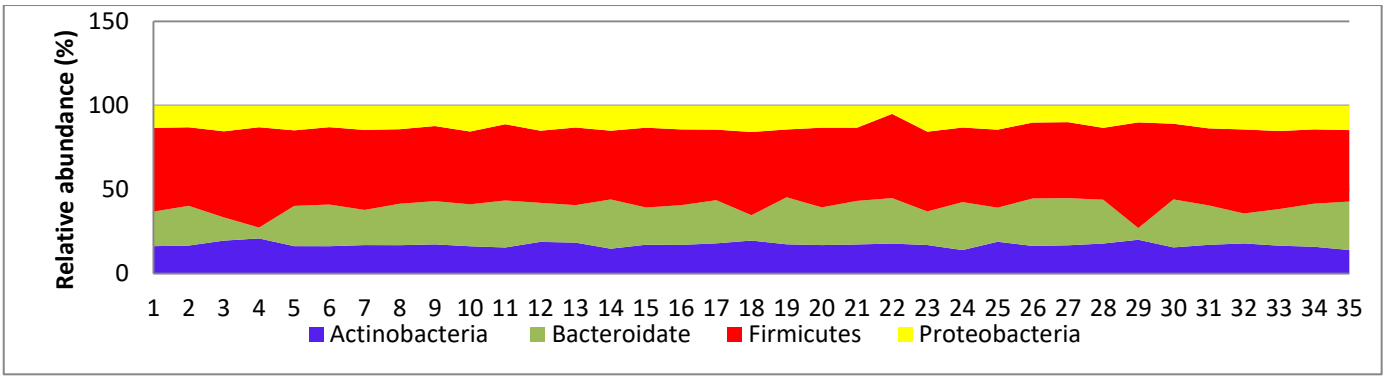


Fig 1 -The % Relative Abundance levels of the four Bacterial Phyla

				Log_{10} genome copies / g wet feces			
Phylum	**Family**	**Genus / Species**	**Detection, %**	**Average±SD**	**25th, 50th, 75th**	**Adults ***	**Average/Adults * 100**
Actinobacteria	**Bifidobacterium**	**Bifidobacterium**	**100**	10.0±**0.15**	**9.44; 10.18; 10.68**	7.2 ± 1.7	**> 100**
Bacteroidetes	**Bacteroidaceae**	**Bacteroidefragilis**	**94**	**9.45±0.24**	**8.43; 9.47; 10.79**	8.1 ± 0.5	**>100**
	Prevotellaceae	**Prevotella group**	**97**	**6.43±0.30**	**5.00; 6.38; 7.03**	**9.0 ± 1.1**	**71.4**
	Lactobacillaceae	**Lactobacillus spp**	**100**	**8.01±0.21**	**7.13; 7.81; 8.99**	**8.3 ± 0.3**	**96.5**
Firmicutes	**Cl. cluster IV**	**Clleptum group**	**100**	**9.96±0.15**	**9.33; 10.05; 10.67**	9.9 ± 0.6	**> 100**
	Ruminococcaceae	**Faecalibacteriumpraunitzii**	**100**	**9.7±0.12**	**9.12; 9.83; 10.33**	9.4 ± 0.3	**> 100**
Proteobacteria	**Enterobacteraceae**	**Enterobacteriaceae**	**100**	**8.37±0.22**	**7.70; 8.56; 9.21**	9.4 ± 0.3	**89**

Table 2 presents the abundance of the 7 studied bacterial taxa in the faeces of 35 children

Table (2) presents the abundance of the 7 studied bacterial taxa in the faeces of 35 children. Five taxa were detected in all children, whereas the Bacteroides and Prevotella groups were detected in 94 and 97% of the child population, respectively. The Actinobacteria phylum was represented by the most abundant Bifidobacterium genus, averaging 7.4±1.7 log10 genome copies/g faeces, and members of this genus were the most frequently encountered taxa in all children. The faecal profile was also rich in the Clostridium leptum group and Faecalibacterium praunitzii. F Boxes indicate 25th to 75th percentiles, with mean relative abundances marked as lines and whiskers indicating the range (minimum/maximum) from the boxes. With regard to age group, the mean genome copies of Bifido- bacterium spp. were higher in younger children ≤5 years than among their older mates >5 years. Enterobacteriaceae behaved in a similar manner. The genome copies of Faecalibacterium praunitzii were significantly higher in children >5 years old than in those ≤5 years old. In boys, the genome copies of two taxa, Clostridium leptum and Faecalibacterium, were significantly higher (P<0.05) than their respective genome copies in girls.

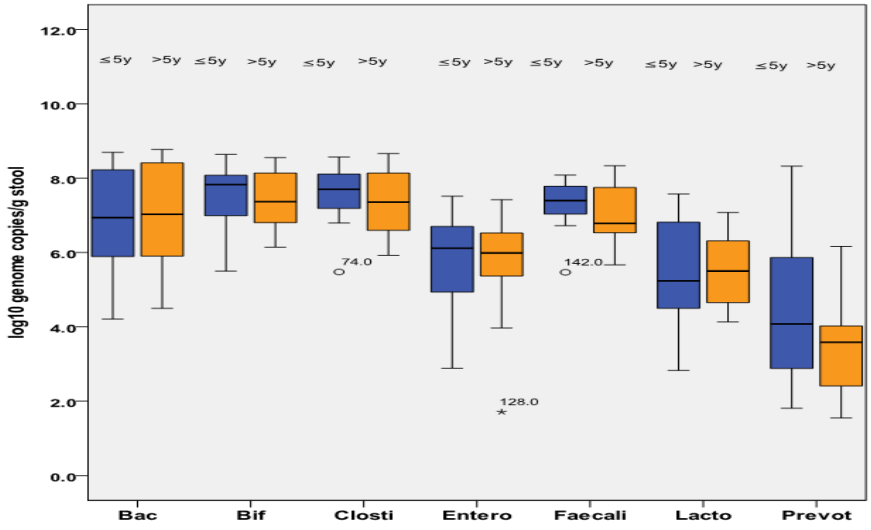
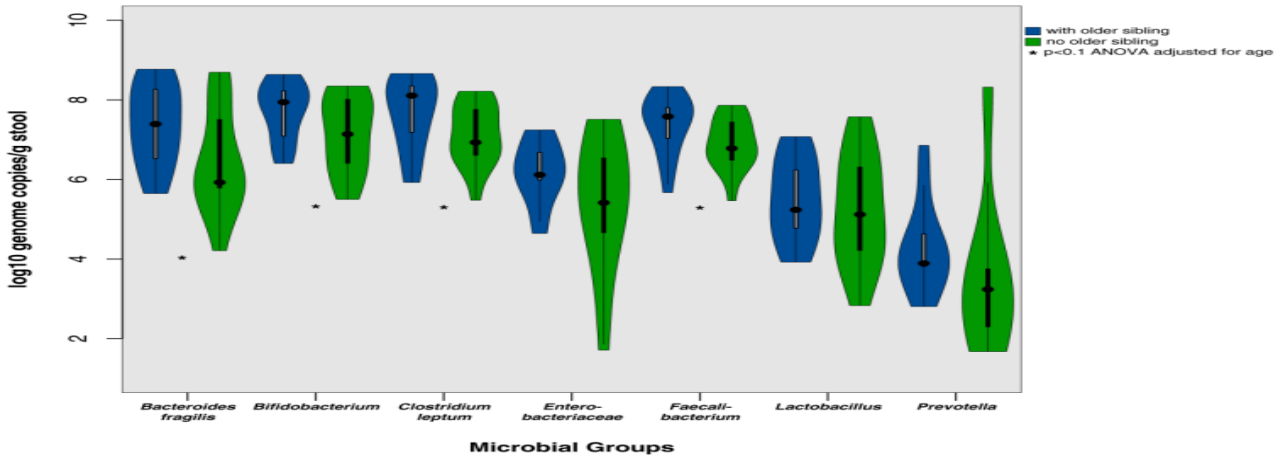


Figure 2 illustrates the Whisker box plots of the studied taxa classified according to age group and sex.

The presence of older siblings cohabiting within the same household was associated with significantly higher faecal bacterial counts of Bacteroides, Bifidobacterium, Clostridium leptum and Faecalibacterium in the respective younger brothers and sisters (P<0.05) compared with those from unrelated families (Figure 3).



The diversity in Bifidobacterium between brothers and sisters from the same family was lower than the respective diversity among unrelated children,suggesting transmission of a number of Bifidobacterium strains across family members (Figure 4).

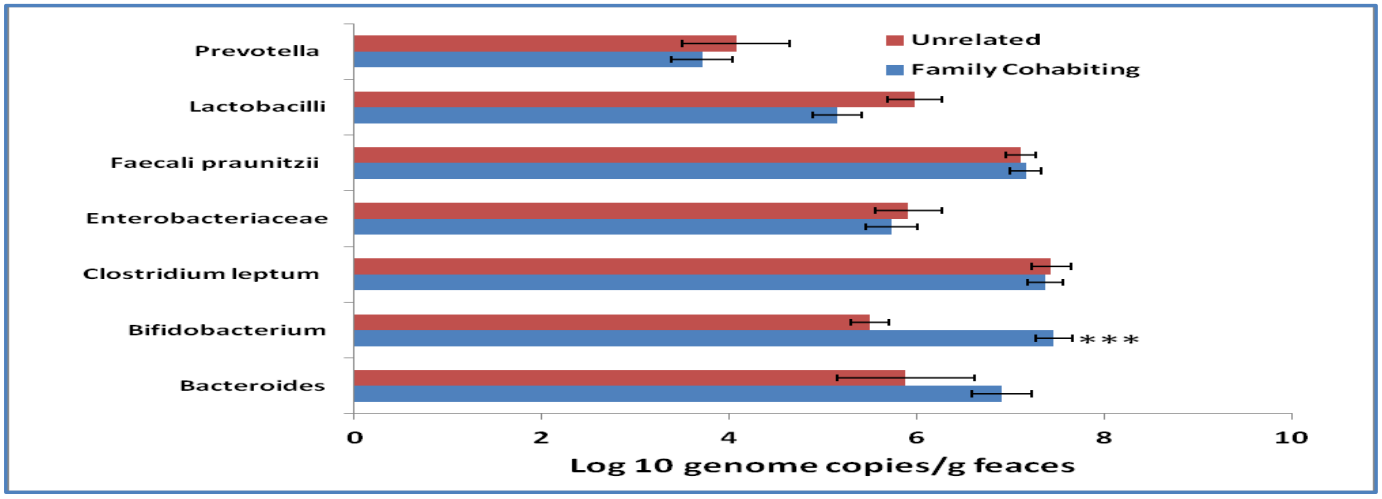


Fig 4- Transmission of a number of Bifidobacterium strains across family members

Multivariate analysis of the confounding effect of familial cohabitation (11 families versus 10 unrelated children) and the presence of an older sibling within the same family on bacterial taxa after age adjustment showed a modest significant difference (0.1<P>0.05). Figure (5 is a dendrogram for the complete linkage hierarchical clustering of the faecal samples from families cohabiting brothers and sisters versus unrelated children. Despite many taxa being shared between children, their distribution differed substantially.

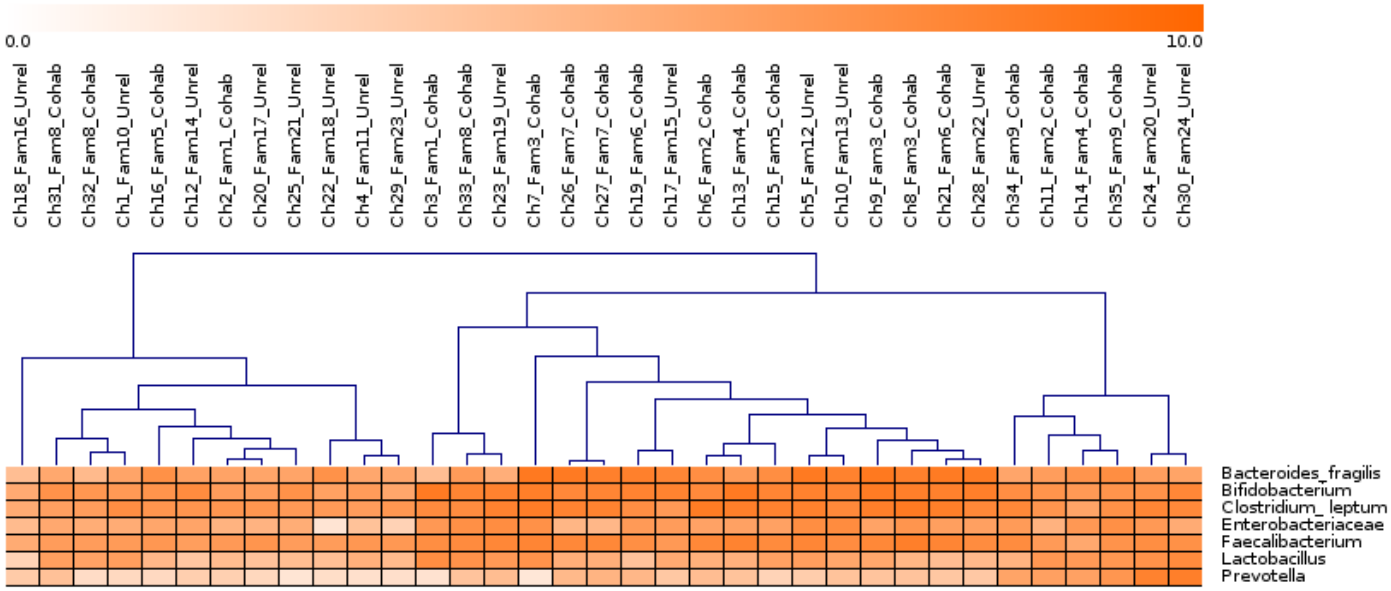


Fig.5-Dendrogram for the complete linkage hierarchical clustering

6. Discussion

The early paediatric period shapes the composition of the gut microbiota depending on infant transitions, which remain relatively stable in adulthood. Accordingly, a healthy host–microorganism balance must optimally perform metabolic and immune functions and prevent disease development [45]. Species richness is a major marker for gut health because high bacterial richness and diversity reflect ecosystem stability and resilience [46]. The identification of a dominant set of phylotypes and core microbiome bacterial taxa shared by the majority (≥ 95%) of healthy children is extremely important and highly warranted [29,34,47]. The field of microbiome research has evolved rapidly over the past few decades and is driven by highly sophisticated technology development,so-called multi-omics [48]. High-throughput isolation(culturomics) of gut bacteria followed by MALDI-TOF mass spectrometry with an optimized database [49] succeeded in the identification of 174 (45%) bacterial species in the human gut [50]. Currently, an estimate of 386 bacterial species are the global human gut microbiome and have been identified mainly by culture-independent next-generation sequencing targeting bacterial 16S. The % relative abundances of the phyla Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria of 23:46:17:12,

respectively (Figure 1), differ from the % relative abundances of 63.7:22:10.1:0.8 reported among BurkinoFaso children and 27.3:52:7:6.7 reported among Italian children [51]. The Firmicutes/Bacteroidetes ratio averaged 1.52±0.19 in the present study, differing from the respective ratios of 0.47 ± 0.05 reported among BurkinoFaso children [51] and 0.69 (0.39–1.32) among Ukrainia children [52]. The F/B ratio increased linearly from 2.6 ±1.83 to 7.7±7.1 among lean and obese Italian children, respectively [53], but the large variation in the ratio makes it an inappropriate robust marker for obesity. At the weaning period, the abundance of Bacteroides spp. Increases [54]; these bacteria break down complex xylan, mannan and xyloglucan and produce the SCFA propionate [50]. The phylum Firmicutes is represented by the gram-positive bacterium Faecalibacterium prausnitzii of the family Ruminococcaceae, which was abundant in the faeces of our children, reflecting a healthy gut [22] because a heavy load of pathogenic microbes led to its depletion [55]. Faecalibacterium prausnitzii ferments dietary fibre, producing the SCFA butyrate via the butyryl-CoA pathway [56]. Butyrate is a health-promoting molecule because it is a fuel source for enterocytes and colonocytes [57]; it increases mucin synthesis [54], which changes bacterial adhesion [58]; it plays an important role in the maintenance of gut barrier function [59,60]; it is an anti-inflammatory agent and inhibits the activation of the transcription factor NF-κB [61]. The high abundance of Lactobacilli spp. in the faeces of present children is also an indicator of a healthy gut [62]. Lactic acid, the fermentation intermediate of lactobacilli, is a cross-feeding substrate for the colonic bacteria Eubacterium hallii to produce either butyrate or propionate [63,64]. Eubacterium hallii accounts for 2 – 3% of the human faecal community, and its capacity for the production of the SCFAs butyrate and propionate via metabolic cross-feeding lead to lowering the pH of the colonic milieu to approximately 5.5. At this pH, the growth of colonic Bacteroides spp. is selectively inhibited, and members of the Firmicutes phylum become dominant because they are not sensitive to acidic pH values [65]. Actinobacteria, represented by Bifidobacterium spp. are gram-positive, rod-shaped, anaerobic commensals, and the 'infant type' Bifidobacterium breve and B. infantis may reach 90% of the gut microbiota of healthy breastfed infants [66-69], with a dramatic decline when a milk-based diet is discontinued [70]. The abundance of Bifidobacterium spp. in 3-year-old children is several-fold higher than that in American adult from the same location [71]. B. bifidum is the only strain capable of metabolizing gut mucins, suggesting that it has a role in adulthood [72]. Swedish children 4 – 5 years old presented with high concentrations of Bifidobacterium, averaging 11.45 genome copies per g of faeces [73]. Japanese school children aged 7 to 11 years also harboured a high abundance of Bifidobacterium and fewer potentially pathogenic bacteria than age-matched mates from other Asiatic countries, suggesting a unique Japanese living environment and dietary pattern [74, 75]. The proportions of Actinobacteria in healthy and malnourished Bangladesh children were 6 and 1%, respectively [76]. No Bifidobacterium could be detected in the gut microbiota of the selected ethnic Guangzhou Chinese group [66]. Bifidobacteria could not be detected in the gut microbiota of Finnish preschool children following the administration of macrolide antibiotics, and the reduced count remained for up to 2 years after the macrolide course [77]. In Germany, the short-term use of broad-spectrum antibiotics abolished members of the genus Bifidobacterium within the 180-day study time frame, and antibiotic resistance gene (resistome) carriage modulated the recovery processes [78]. The maintenance of a healthy gut by Bifidobacteria is due to its effect on reinforcement of the host intestinal barrier, competitive exclusion of pathogens, and modulation of the immune response [79].

The relatively high abundance of Enterobacteriaceae among our children is consistent with our earlier finding indicating a potential higher load of pathogenic microorganisms in the gut of Egyptian children, probably attributed to dietary unhygienic food preparation or other confounding factors[40]. A higher relative abundance of all Proteobacteria species (32%) was reported in the guts of 2- to 3-year-old malnourished Bangladesh children than in their age-matched healthy controls[76]. Latest findings identifies Proteobacteria phyla as possible microbial signature of inflammatory bowel disease but also in extraintestinal diseases including lung diseases, such as asthma and chronic obstructive pulmonary disease [80]. The overall adult-like gut microbiota in the present study was arbitrarily estimated by comparing their abundances with their respective abundances among Egyptian adults (24 years old) [44]. In the last column of table (2), the colonization of the gut microbiota reported among adults was set = 100; the relative colonization of 5 gut bacteria among the studied children varied between 43 and 80% of the respective adult abundances. The abundances of Bifidobacterium spp. and Clostridium groups were exceptions and exceeded 100% of the respective adult values. This finding is consistent with an earlier finding from the US81 and differs from a Dutch study, which indicated that the gut microbiota composition of 7-year-old children showed overall adult-like behavior[82].

The present study showed significant transmission or shared inferred genotypes for Bifidobacterium strains within the gut microbiomes of brothers and sisters cohabiting the same household [Figure 4]. This finding is consistent with a Japanese study, which showed transmission of Bifido longum strains across family members [43] and between selected individual sets of twins cohabitating for decades [83].

The present study showed increased gut microbial richness in children with older siblings (Figure 3), which is attributed to the positive impact of exposure to non-pathogenic microbes with older siblings on increased gut microbial richness and prevention of allergic diseases [84, 85]. Sex hormones were reported to play a role in shaping the gut microbiota composition between males and females at puberty [86] and in young Tanzanian boys and girls with different labours with regard to foraging [87]. The higher abundances of the taxa Clostridium and Faecalibacterium in the faeces of our studied boys compared to their respective abundances in the girls ($P<0.05$) could partially be interpreted as the impact of gender-specific immune differences in the gut microbiota composition between boys and girls [88]. Currently, there is no agreement on the sex differences in gut microbiota composition among the different populations [89].

For the past decade, the scientific community has been blessed with a wealth of knowledge about the gut microbial community structure and function and the specific contributions of geographical differences, race and ethnicity to the variations in the gut microbiome among children [29]. However, data on gut microbiome composition in African and Middle Eastern children are scant except for the work done on children from Burkina Faso [47] and Senegal [31]. Accumulating evidence has shown adverse effects of oral administration of micronutrient powder [MNP] containing a high dosage of iron to African infants and children on the abundances of Bifidobacterium and Lactobacilli in their colon, with increased abundance in pathogenic Enterobacteriaceae [90]. Other drastic effects on the gut included decreased faecal butyrate SCFA production and inflammation of the gut, which led to the development of stable degraded microbiota as a result of persistent stressors [91] and increased

mortality rates. These catastrophic results are typical examples of the risks of advertising and marketing globalized new products without any consideration of the delicate and unique structure of African ancient microbial communities of the gut microbiota. Sophisticated equipment and tools using throughput cultoromics and 16S rRNA gene amplicon sequencing require a well-equipped molecular laboratory and well-trained hands, which are beyond the capacity in low-income countries such as Egypt. Species- specific PCR primers combined with the application of real-time quantitative PCR (qPCR) were used in the present study for the specific detection and quantification of gut microbiota.This study was limited by the small sample size (35 children), which did not allow for enough statistical power to assess associations of interest. Despite these limitations, the samples were collected from a similar age, single geography-economy strata (all children from urban Giza from modest economic strata, which are supposed to be the key factors influencing gut microbiota composition). The number of variables in our analysis was reduced as much as possible. Considering the pilot nature of the study, the results provide a rough picture of the dynamic microbial community. The study describes patterns of the gut microbiota and provides valuable insight into their normal developmental changes among children 3 – 9 years old in this region; thus, it contributes to the limited knowledge of microbial variability in children. This study is replicated in a much larger population with an emphasis on dietary patterns under investigation to explore the structural and functional configurations of the gut microbiota of early school-age children. Such information is crucial for developing new intervention strategies to restore symbiosis to modulate the microbial community and to promote better health at various stages of life.

8. Acknowledgments

We would like to acknowledge all the individuals involved in samples collection and wish to thank all the participating parents for their full understanding and collaboration

9. Funding/Conflicts of Interests

The study was approved by the Medical ethical committee of the NRC: Medical Research Ethics Committee (MREC 13-125).

Conflict of Interest: The authors declare no competing financial interests.

Funding: No formal grants or projects.

10. References

1. DiGiulio DB (2012) Diversity of microbes in amniotic fluid. Semin Fetal Neonatal Med 17(1): 2-11.

2. DiGiulio DB (2015) Prematurity and perinatal antibiotics: A tale of two factors influencing development of the neonatal gut microbiota. J Pediatr 166(3): 515–517.

3. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, et al. (2011) Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A. 108:4578– 4585.

4. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, et al. (2017). The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev 81(4): 00036-17

5.Mackie RI, Sghir A, Gaskins HR (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr 69(5): 1035S–1045S.

6. Chernikova DA, Madan JC, Housman ML, Zain-ul-abideen M, Lundgren SN et al. (2018). The premature infant gut microbiome during the first 6 weeks of life differs based on gestational maturity at birth. Pediatric Res 84(1):71–79.

7. Arboleya S, Sanchez B, Milani C, Duranti S, Solıs G, et al. (2015).Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. J Pediatr 166 (3):538- 544.

8. Bäckhed F, Roswall J, Dahlgren J, Peng Y, Qiang F, et al. (2015).Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 17(5):690–703.

9. Ho NT, Li F, Lee-Sarwar KA, Tun HM, Brown BP, et al. (2018) Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun 9(1):4169.

10. Van den Elsen LWJ, Garssen J, Burcelin R, Verhassel V. (2019) Shaping the gut microbiota by breastfeeding: The gateway to allergy prevention? Front Pediatr 7:47

11. Junick J, Blaut M. (2012) Quantification of human fecal Bifidobacteriumspecies by use of quantitative real-time PCR analysis targeting the groELgene. Appl Environ Microbio 78(8) : 2613–2622.

12. Tanaka M, Nakayama J (2017) Development of the gut microbiota in infancy and its impact on health in later life. Allergol Int 66(4):515- 522.

13. Clarke G, O'Mahony SM, Dinan TG, Cryan JF (2014) Priming for health: gut microbiota acquired in early life regulates physiology, brain and behavior. Acta Paediatr 103(8):812–819.

14. Fitzstevens JL, Smith KC, Hagadorn JI, Caimano MJ, Matson AP, et al (2017) Systematic review of the human milk microbiota. Nutr Clin Pract 32(3):354- 364.

15. Gómez-Gallego C, Morales JM, Monleón D, du Toit E, Kumar H, et al. (2018) Human breast milk NMR metabolomic profile across specific geographical locations and its association with the milk microbiota. Nutrients. 10(10):1355

16. Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, et al. (2017) Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. JAMA Pediatr 17(7):647–654.

17. Guaraldi F, Salvatori G (2012) Effect of breast and formula feeding on gut microbiota shaping in newborns. Front Cell Infect Microbiol 2:94.

18. Nagpal R, Tsuji H, Takahashi T, Nomoto K, Kawashima K, et al.(2017). Ontogenesis of the gut microbiota composition in healthy, full-term, vaginally born and breast-fed infants over the first 3 years of life: a quantitative bird's-eye view. Front Microbiol. 8: 1388

19. Ringel-Kulka T, Cheng J, Ringel Y, Salojärvi J, Carroll I, et al. (2013) Intestinal microbiota in healthy U.S. young children and adults—a high throughput microarray analysis. PLoS One. 8(10):e64315.

20. Wang M, Li M, Wu S, Lebrilla C, Chapkin RS, et al. (2015) Fecal microbiota composition of breast-fed infants is correlated with human milk oligosaccharides consumed. J Pediatr Gastroenterol Nutr. 60(5): 825–833.

21. Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. (2016) Bifidobacteria and butyrate-producing colon bacteria:Importance and

strategies for their stimulation in the human gut. Front. Microbiol., 7:979-999.

22. Rajilić-Stojanović M, Smidt H, de Vos WM. (2007) Diversity of the human gastrointestinal tract microbiota revisited. Environ Microbiol. 9(9):2125-2136.

23.Laursen MF, Andersen LBB, Michaelse KF, Mølgaard C, Trolle E et al. (2016) Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. mSphere 1(1):e00069-15

24. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, et al.(2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 107(33):14691–14696.

25. Soldi S, Vasileiadis S, Lohner S, Uggeri F, Puglisi E et al (2019). Prebiotic supplementation over a cold season and during antibiotic treatment specifically modulates the gut microbiota composition of 3-6 year-old children. Benef Microbes.10:253-263.

26. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA.(2016) A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility.Cell 167(5):1339–1353.

27. Sicard J-F, Le Bihan G, Vogeleer P, Jacques M, Harel J. (2017) Interaction of the intestinal bacteria with components of the intestinal mucus. Front Cell Infect Microbiol. 7:387.

28. Faith JJ, Guruge JL, Charbneau M, Subramanian S, Seedorf H, et al. (2013) The long term stability of the human gut microbiota. Science 341(1614): 1237439.

29.Valles Y, Artacho A, Pascual-Garcia A, Ferris ML, Gosalbes MJ, et al (2014). Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. PLoS Genet 10(6): e1004406.

30. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, et al. (2014). Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 510(7505):417–421.

31. Alou MT, Million M, Traore SI, Mouelhi D, Khelaifia S, et al. (2017). Gut bacteria missing in severe acute malnutrition, can we identify potential probiotics by culturomics? Front Microbiol 8:899.

32. Raman AS, Gehrig JL, Venkatesh S, Chang H-W, Hibberd MC, et al. (2019) A sparse covarying unit that describes healthy and impaired human gut microbiota development.Science 365(6449): eaau4735.

33. Million M, Diallo A, Raoult D.(2017) Gut microbiota and malnutrition. Microb Pathog. 106: 127-138.

34. Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, et al.(2015). Structure and function of the healthy pre-adolescent pediatric gut microbiome. Microbiome 3:36 – 48.

35. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, et al. 2012) Human gut microbiome viewed across age and geography. Nature 486 (7402):222–227.

36. Berding K, Holscher HD, Arthur A, Donovan S. (2018) Fecal microbiome composition and stability in 4- to 8 year old children is associated with dietary patterns and nutrient intake. J Nutr Biochem 56:165-174.

37. Avershina E, Storrro O, Oien T, Johnson R, Pope P, et al.(2014) Major fecal microbiota shifts in composition and diversity with age in a geographically restricted cohort of mothers and their children. FEMS Microbial Ecol 87:280 – 290.

38. De Meij TGJ, Budding AE, de Groot EFJ, Jansen FM, Kneepkens CM, et al (2019) Composition and stability of intestinal microbiota of healthy children within a Dutch population. FASEB J 30(4) 1512 – 1522.

39. Li J, Jia H, Cai X, Zhong H, Feng Q, et al. (2014) An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol, 32(8): 834–841.

40. Shankar V, Gouda M, Moncivaiz J, Gordon A, Reo NV, et al. (2017). Differences in gut metabolites and microbial composition and functions between Egyptian and U.S. children are consistent with their diets. mSystems, 2(1): e00169-16.

41. Stecher B. (2015) The roles of inflammation, nutrient availability and the commensal microbiota in enteric pathogen infection. Microbiol Spectrum, 3(3): MBP-0008-2014.

42. Franzosa EA, Huang K, Meadow JF, Gevers D, Lemon KP et al. (2015) Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci U S A 112(22) E2930–E2938.

43. Odamaki T, Bottacini F, Kato K, Mitsuyama E, Keisuke Y et al (2018) Genomic diversity and distribution of Bifidobacterium human lifespan. FEMS Microbiol Rev. 44(3): 369–385

44. Labib E, Blaut M, Hussein L, Gouda M, Kramer DL, et al.(2018). Molecular diversity of gut microbiota and short chain fatty acids in Egyptian adults following dietary intervention with fermented sobya. J Food Microbiol Saf Hyg 3: 139.

45. Rinninella E, Raoul P, Cintoni M , Franceschi F, Miggiano GAD, et al. (2019).

What is the healthy gut microbiota composition? A changing ecosystem across age,

environment, diet, and diseases. Microorganisms 7(1):14.

46. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, et al. (2016) Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. Gut 65(1): 57–62.

47. Kisuse J, La-ongkham O, Nakphaichit M, Therdtatha P, Momoda R, et al. (2018) Urban diets linked to gut microbiome and metabolome. Frontiers Microbiol. 9 :1345 – 1360.

48. Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-C C, et al. (2020). Microbiome definition re-visited: old concepts and new challenges. Microbiome 8(1):103.

49. Lagier J-C, Hugon P, Fournier P-E, LaScola B, Raoult D. (2015) The rebirth

of culture in microbiology through the example of culturomics

to study human gut microbiota. Clin Microbiol Rev 28(1): 237-263.

50.Thursby E, Juge N. Introduction to the human gut microbiota (2017). Biochem J 474(11): 1823-1836.

51. De Filippo C, Di Paola M, Ramazzotti M, Albanese De, Pieraccini G.(2017) Diet, environments, and gut microbiota. A preliminary

investigation in children living in rural and urban Burkina Faso and Italy. Front. Microbiol, 8:1979- 1992.

52. Vaiserman A , Romanenko M, Piven L, Moseiko V, Lushchak O,et al. (2020).

Differences in the gut Firmicutes to Bacteroidetes ratio across age groups in healthy

Ukrainian population. BMC Microbiology 20:221.

53. Riva A, Borgo F, Lassandro C, Verduci E, Morace, G, et al. (2017) Pediatric obesity is associated with an altered gut microbiota and discordant shifts in Firmicutes populations. Environ Microbiol.19(1): 95–105.

54. Niu J, Xu L, Qian Y, Sun Z, Yu D, et al.(2020) Evolution of the gut microbiome in early childhood: A cross-sectional study of Chinese children. Front. Microbiol., 11:439.

55. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M. (2017) Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. ISME J 11: 841–852.

56. Liu H, Wang J, He T, Becker S, Zhang G,et al. 2018) Butyrate: A double-edged sword for health? Adv Nutr 9(1): 21–29.

57. Heinken A, Khan T, Paglia G, Rodionov D A, Harmsen HJM. (2014) Functional metabolic map of Faecalibacteriumprausnitzii, a beneficial human gut microbe. J Bacteriol 196 : 3289–3302.

58. Jung T-H, Park JH, Jeon W-M, Han K-S. (2015). Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway. Nutr Res Pract 9(4): 343 – 349.

59. Knudsen KEB ,Lærke HN, Hedemann MS, Nielsen TS , Ingerslev AK, et al. (2018). Impact of diet-modulated butyrate production on intestinal barrier function and inflammation. Nutrients 10(10):1499.

60. Wang H-B, Wang P-Y, Wang X, Wan Y-L, Liu Y-C. (2012). Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1transcription. Dig Dis Sci 57(12): 3126- 3135.

61. Venegas DP, De la Fuente MK, Landskron G, González MJ, Quera R, et al. (2019) Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Frontier Immunol 10:277.

62. Goldin BR, Gorbach SL, SaxelinM, Barakat S, GueltiriL, et al. (1992). Survival of Lactobacillus species (strain GG) in human gastrointestinal tract. Dig Dis Sci 37:121 -128.

63. Duncan SH, Louis P, Flint HJ. (2004). Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol 70(10): 5810–5817

64. Engels C, Ruscheweyh H-J, Beerenwinkel N, Lacroix C, Schwab C. (2016). The common gut microbe Eubacteriumhallii also contributes to intestinal propionate formation. Front. Microbiol 70:713..

65. Louis P, Flint HJ.(2017) Formation of propionate and butyrate by the human colonic microbiota. Environ Microbiol. 19(1):29–41.

66. Cionci NB, Baffoni L, Gaggìa F, Di Gioia D (2018) Therapeutic microbiology: The role of Bifido- bacterium breve as food supplement for the prevention/treatment of paediatric diseases. Nutrients 10(11): 1723-1749.

67. Khine WW T, Zhang Y, Goie GJY, Wong MS, Liong M, et al (2019). Gut microbiome of pre-adolescent children of two ethnics residing in three distant cities. Sci Rep 9:7831.

68. Ruiz-Moyano S, Totten SM, Garrido DA, Smilowitz J T, German B et al. (2013) Variation in consumption of human milk oligosaccharides by infant gut-associated strains of Bifidobacteriumbreve. Appl. Environ Microbiol 79(19), 6040–6049.

69. Turroni F, Milani C, Duranti S, Mahony J, van Sinderen D3, et al (2018) Glycan utilization and cross-feeding activities by bifidobacteria. Trends Microbiol 26:339–350.

70. Khonsari S, Suganthy M, Burczynska B et al. (2016). A comparative study of bifidobacteria in human babies and adults. Biosci Microbiota Food Health 35(2): 97–103.

71. Ringel-Kulka T, Cheng J, Ringel Y, Salojärvi J, Carroll I, et al. (2013). Intestinal microbiota in healthy U.S. young children and adults- a high throughput microarray analysis. PLoS One, 8: e64315.

72. Ruiz L, Delgado S, Ruas-Madiedo P, Sánchez B, Margolles A (2017). Bifidobacteria and their molecular communication with the immune system. Frontier Microbiol. 8: Article 2345.

73. Karlsson CLJ, Önnerfält J, Xu J, Molin G, Ahrné S. (2012). The Microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 20(11) 2257 – 2261.

74. Nakayama J (2019). Health status of Japanese and Asians indicated by gut microbiome research. J Integrated Study Dietary Habits 29:137-140.

75. Nishijima S, Suda W, Oshima K, Kim S-W, Hirose Y, et al. (2016) The gut microbiome of healthy Japanese and its microbial and functional uniqueness. DNA Res. 23(2): 125–130.

76. Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, et al. (2011) Gut microbiota of healthy and malnourished children in Bangladesh. Front Microbiol. 2:228.

77. Korpela K, Salonen A, Virta LJ, Kekkonen RA, Forslund K. (2016) Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nat Commun.7: 10410.

78. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, et al. (2018). Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Commun.3(11): 1255–1265.

79. Castanys-Muñoz E, Martin MJ, Vazquez E. (2016). Building a beneficial microbiome from birth. Adv Nutr, 7(2):323–330.

80. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A (2017). Proteobacteria: A common factor in human diseases. BioMed Res Intern

9: 1- 7. Article ID : 9351507.

81. Cheng J, Ringel-Kulka T, Heikamp-de Jong I, Ringel Y, Carroll I, et al. (2015). Discordant temporal development of bacterial phyla and the emergence of core in the fecal microbiota of young children. ISME J. 10: 1002– 1014.

82. Derrien M, Alvarez A-S, deVos WM (2019). The gut microbiota in the first decade of life. Trends Microbiol 27(12) : 1-14.

83. Koo H, Hakim JA, Crossman DK, Lefkowitz EJ, Morrow CD. Sharing of gut

microbial strains between selected individual sets of twins cohabitating for decades. PLoS ONE 2016, 14: e0226111.

84. Laursen MF, Zachariassen G, Bahl MI, Bergström A, Høst A, et al. Having older siblings is associated with gut microbiota development during early childhood.

BMC Microbiol 2015, 15: Article number:154. doi. 10.1186/s12866-015-0477-6.

85. Hasegawa K, Linnemann RW, Mansbach JM, Ajami NJ, Espinola JA, et al.

Household siblings and nasal and fecal microbiota in infants. Pediatrics.59: 473–481.

86. Gao X, Zhang M, Xue J, Huang J, Zhuang R, Zhou X, et al. Body mass index

differences in the gut microbiota are gender specific. Front Microbiol 2018, 9:

1250.doi.org/10.3389/ fmicb.2018.01250.

87. Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, et al. (2014).

Gut microbiome of the Hadza hunter-gatherers. Nat Commun. 2014; 5: 3654.

88. Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, et al. The

impact of gut microbiota on gender-specific differences in immunity. Front.

Immunol. 2017, 8:754.

89. Kim YS ,Unno T, Kim B-Y , Park M-S. Sex differences in gut microbiota. World J

Mens Health. 2019; doi.org/10.5534/wjmh.190009. doi. Org. /10.5534/wjmh.190009.

90. Dostal A, Baumgartner J, Riesen N, Chassard C, Smuts CM, Zimmermann MB,

Lacroix C. Effects of iron supplementation on dominant bacterial groups in the gut,

faecal SCFA and gut inflammation: a randomised, placebo-controlled intervention

trial in South African children. Br J Nutr 2014,112: 547–556.doi:10.1017/

S0007114514001160.

91. Blanton LV et al. Gut bacteria that prevent growth impairments transmitted

by microbiota from malnourished children. Science 2016, 351: 6275.doi. 10.1126/

science.aad3311.

92. Matsuki T, Watanabe K, Fujimoto J, Takada T, Tanaka R (2004). Use of 16S rRNA

gene- targeted group-specific primers f,or real-time PCR analysis of predominant

bacteria in human feces. Appl Environ Microb 2004, 70: 7220-7228. doi.10.1128/

AEM.70.12.

93. RinttiläT, Kassinen A, Malinen E, Krogius L, Palva A (2004). Development of an

extensive set of 16S rDNA-targeted primers for quantification of pathogenic and

indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol.

94:1166–1177.

94. Bartosch S, Fite A, Macfarlane GT, McMurdo ME (2004) Characterization of

bacterial communities in feces from healthy elderly volunteers and hospitalized

elderly patients by using real-time PCR and effects of antibiotic treatment on the

fecal microbiota. Appl Environ Microbiol 70:3575–3581.

95. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG,

et al. (2008). Faecalibacterium prausnitzii is an anti-inflammatory commensal

bacterium identified by gut microbiota analysis of Crohn disease patients.

Proc Natl Acad Sci 105: 16731-16736.