

The Effects of Gut Microbiota on Childhood Obesity: A Systematic Review and Meta-Analysis

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Abstract

Purpose: Over the past decades, the gut microbiome had been associated with obesity. Therefore, the objective of this review is to analyze the association between gut microbiota and childhood obesity, and to determine the factors that affect the gut microbiota. **Methods:** A systematic review was performed using three major search databases. Data were searched using PubMed, Scopus and Cochrane Library. Twenty-three (23) articles since 2015 to 2019 were selected for the qualitative synthesis. Three (3) were included for meta-analysis on the predominant taxa in children with obesity. **Results:** Participants range from 22 to 1087, among 0 to 18 years old. Bacteroidetes showed consistent inverse association with BMI (pooled OR 0.74(95% CI;0.42,1.19). The microbiome from the firmicutes phylum showed inconsistent association with obesity. Among the factors effecting the microbiomes include diet type, antibiotic exposure, breastfeeding, pregnancy weight gain, and home disinfectants. **Conclusion:** The dynamic changes of microbiomes composition were associated with adiposity among children since birth. Bacteroidetes phylum should be further researched for its potential protective mechanism. Besides, further understanding on the factors effecting gut microbiome will improve the preventive effort to combat childhood obesity.

Keywords: Bacteroidetes, Childhood obesity, Gut microbiota, Microbiome.

INTRODUCTION

Childhood obesity is an important public health issue. Globally, it is estimated in 2016 that the number of overweight children under the age of five is found to be over 41 million [1]. Almost half of all overweight children under 5 lived in Asia and one quarter lived in Africa¹. As a developing Asian country, Malaysia is also experiencing a rapidly rising prevalence of childhood Children obesity, consistent with international trends [2]. In a Malaysian cross-sectional study, prevalence of overweight and obesity amongst children aged 6 to 12 years increased from 20.7% in 2002 to 26.4% in 2008 [3]. A nationwide study published in 2013 found that approximately 34.5% of urban primary school-aged children were either overweight or obese [4].

Early prevention of childhood obesity is important because overweight children and adolescents are at greater risk for health problems compared with their normal-weight counterparts and are more likely to become obese adults [5]. In

addition to consequences later in life, obese children and adolescents are more likely to have serious health conditions, such as cardiovascular, metabolic, and psychosocial illnesses; type 2 diabetes; hypertension; high cholesterol; stroke; heart disease; nonalcoholic fatty liver disease; certain cancers; and arthritis [6,7]. Moreover, other reported health consequences of childhood obesity include eating disorders and mental health issues, such as depression and low self-esteem [8].

Recently, there is increasing interest in the role of gut microbiota as risk factor for childhood obesity. There are various studies that show association between gut microbiota and obesity [9–11]. In particular, the composition of gut microbiota known to play a role concerning obesity. By definition, gut microbiota is considered as an assortment of microorganisms that inhabit the gastrointestinal tract. It is known that most of the human's populations microbiota is composed by 5 phyla Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia [12]. Bacteroidetes and Firmicutes consist of around 90% of the total bacterial species [12,13]. The composition of this microbial community depends on the host, but it can also be modified by

exogenous and endogenous events [14]. Among the factors that are thought to influence gut microbiota include maternal health and microbiota composition, birth mode, antibiotic exposure, breast milk, prebiotics or/and probiotics, type of diet and level of physical activity [15–17].

It is also found that the composition of the bacterial diversity seems to change between lean and obese, increasing the number of Firmicutes to the detriment of Bacteroidetes [11,12,18,19] in obese patients and also in type 2 diabetes, which is pathology in close relationship to obesity. However, recent studies have shown controversial results [20,21]. Studies of association between microbiota profiles and different phenotypes or body mass index have found positive and negative associations among the different phyla that populate the intestines. Latest advances in sequencing of specific regions of 16S or 18S ribosomal genes allows the identification of organisms and their relative abundance in purified DNA [22].

Thus, a systematic review was conducted to fulfil the following objectives: 1) To analyze the association between gut microbiota composition and childhood obesity and 2) To determine the factors associated with gut microbiota in childhood obesity.

METHODOLOGY

Data were searched using PubMed, Scopus and Cochrane Library on 1st April 2019 with the search term; Childhood OR Children OR P*ediatric AND "gut microbiota" OR "gut microbiome" AND (Obesity OR Overweight) AND (Effect OR "Risk factor" OR Cause OR Risk OR Exposure OR associated OR factor). The inclusion criteria for the study selection are; 1) Clinical trial 2) Observational studies 3) article from 2015 to 2019 4) Human Studies. The exclusion criteria for this review are; 1) Review, 2) Animal studies 3) 4) population involve subject more than 18 years old 5) No full article, 6) Not in English 7) Study protocol or ongoing trials. 8) Not related to childhood obesity 9) Not related to gut microbiome.

At the initial stage, three authors performed the selection of potential papers to be included in the study. This is divided into three phases which are analysis of the titles, abstracts and whole text for the relevance of the article to be included in the review. The article selection was done based on the PRISMA study flow diagram [23] as outlined in Figure 1. Next, each selected article will be critically reviewed and appraised by two reviewers. At the disagreement point, the third author will be consulted.

Data extraction were done by all the authors independently using a predetermined data collection form. The data was then crossed checked by all three authors to minimize errors. The Newcastle-Ottawa Quality Assessment [24] was used to assess the quality of the evidence. For studies met at least five criteria out of nine criteria were considered high quality and proceed to meta-analysis. Results was calculated using risk ratio with 95% confident interval. A p value less than 0.05 is considered significant in this study. Statistical heterogeneity was assessed by inspecting the forest plots for overlapping confidence intervals, applying the Chi² test (P value < 0.10 considered to be statistically significant), and the I² statistic (I² value of 50% used to denote moderate levels of heterogeneity). The subgroup

analysis was not performed due to scarce data available for the meta-analysis. We use the random effect model due to the high heterogeneity. Funnel plot is used to assess for assessment of publication bias by plotting the effect size and trial size. Data synthesis were analyzed using Review Manager 5.2 application.

RESULT

The internet-based search

PubMed, Scopus and Cochrane searches identified 144 articles. 20 duplicates were removed. By screening and reviewing for title and abstract, 45 potentially relevant articles were identified and retrieved for more detailed evaluation. Out of these 45 articles, 23 articles fulfilled all the inclusion and exclusion criteria and subsequently 22 articles were excluded with reasons. There were 5 articles rejected because not related to childhood obesity, 1 review article, 6 articles are not studying on gut microbiome, 2 articles are animal studies, 1 not in English, and 5 articles did not have full text available and 2 articles involve participants more than 18 years old. For the meta-analysis, only 3 articles measuring the odds ratio in order to estimate pool effect size.

Description of the Included Studies

23 independent studies were included in this review. In term of study designs, 4 articles were control trials, 11 were cohort studies, 1 case control and 7 cross sectional studies. Most of the studies were conducted in the high-income countries (13 studies), 9 studies were conducted in upper middle-income countries and one from the lower income country. The details of the study characteristics, findings and quality grading are summarized in Table 1.

Gut Microbiota Composition and Childhood Obesity

The phylum Bacteroidetes is composed of three large classes of Gram-negative, non-spore forming, anaerobic or aerobic, and rod-shaped bacteria that are widely distributed in the environment, including in soil, sediments, and sea water, as well as in the guts and on the skin of animals. This phylum is one of the major phyla in the human gut. The highest prevalence recorded is thirty percent (30%) of overall gut microbiota [25]. Three studies found that there is inverse relationship between Body Mass Index (BMI) and Bacteroidetes abundance [26–28]. Two studies found that Bacteroidetes bacteria were reduced in obese children [29,30]. The Peptostreptococcae U. were found to have lower abundance in obese children, [31] and *Povetella* spp were found lower in obese children with high fat diet [32]. Meanwhile there are increase in the bacterial abundance (*B. Plebius* species and parabacteroidetes) in normal weight children, [33–35] and *Provetella* spp. were increased in children with fibre diet intake [36].

The effect of the Bacteroidetes as predominant taxa were analysed using random effect model and presented on the forest plot in figure 2. The Bacteroidetes abundance were found to be a protective factor with pool odd ratio of 0.71(95% CI; 0.42,

1.19). However, this analysis is not statistically significant ($p=0.2$). This could be due to low statistical power in the analysis which contributed by small sample size. The funnel plot in figure 3 showed that the publication bias is not suggestive because the plot is within the funnel line.

For the microbiomes under phylum Firmicutes, the association is genus or species specific. This is because some of the bacteria has positive correlation with obesity and some has negative association with the obesity. In general, the Firmicutes are a phylum of bacteria, most of which have gram-positive cell wall structure. Firmicutes were found in higher abundance in the obese children.^{30,37} Among the genus or species under firmicutes phylum that have positive correlation with high BMI are Dorea (Firmicutes; Clostridiales), Streptococcus (Firmicutes; Lactobacillales), Erysipelotrichaceae (Firmicutes; Erysipelotrichi) Roseburia (Firmicutes; Clostridiales), Lachnospiraceae (Firmicutes; Clostridiales), Ruminococaceae and Aneotruncus [31,35,38]. In contrast, the Ruminococcus (Firmicutes; Clostridia) is inversely related with obesity [31]. The Lactobacillus paracasei ssp were found to be increased during weight reduction, [35] and did not modulate body composition, growth or any of the assessed metabolic markers at school age who were given feeding containing the probiotics LF19 during their infancy.

Bifidobacterium is a genus of gram-positive, nonmotile, often branched anaerobic bacteria from the Actinobacteria phylum. High Bifidobacterium at later age had lower adiposity at 18 months of age [39]. The bacteria are also increasing during weight reduction and due to dietary oligosaccharide from plant and milk and is used as an indicator of healthy condition among obese people [35]. Another species that was found to be low in obesity is Akkermansia from Verrucomicrobia; Verrucomicrobiales phyla.

Goffredo et al. found that the Firmicutes Bacteroidetes Ratio (F/B) has positive correlation with obesity and hepatic fat.²⁶ Besides, F/B ratio were increased in obese subject, and among subject with high fat diet intake [32,35]. Two studies found that imbalance in F/B ratio has correlation with the obesity [27]. However, Karvonen et al and Lopez-Conteras et al found that F/B ratio were not associated with overweight or obesity [31,40].

Factor Affecting Gut Microbiota in Children

Factors affecting the gut microbiota can be categorized into four categories which are diet, breastfeeding practices, pregnancy related, and environmental or other factors. The detail of the association is summarized in Table 2 based on the finding in the included studies.

DISCUSSION

The results of our systematic review and meta-analyses have shown that some microbiota from phylum such as Bacteroidetes, Firmicutes and their composition, measured by F/B ratio do have some effects on causing childhood obesity. This is in agreement

with accumulating evidence showing the importance function of gut microbiota which may be species or strain specific in regulating the adiposity in human. However, to date, many studies in metagenomics are conducted at genus or higher taxonomic levels. This is due to the methodological limitation of assembling individual bacterial genomes directly from metagenomic data [41].

The strength of this review lies on the study population that covered all stages, beginning from conception to adolescents that can contribute to the development of childhood obesity. This includes the intrauterine stage, neonatal period, early infancy, toddlers and school children. One of the important findings from the longitudinal studies conducted was dynamic changes of the microbiome abundance, which were observed since birth. This change causing the dysbiosis of the microbiome in the gut and is associated with adiposity. Besides, metabolic diseases such as obesity are often associated with dyslipidaemia, hypertension, and glucose homeostasis disorders, and are also reported to be associated with intestinal dysbiosis. Dysbiosis is a type of intestinal metagenome and accumulated microbial activity, as well as a combination of genetic factors and classical environment, can promote the development of metabolic disorders [39].

Furthermore, several structural patterns of the gut microbial community have been suggested to be associated with obesity, such as a high Firmicutes/Bacteroidetes ratio and low gene richness. However, the specific relevant members of the gut microbiota and their functional interactions that contribute to obesity development and associated metabolic deteriorations remain elusive [42,43].

Factors affecting gut microbiota

High fat western diet

It is known that high fat intake increases bile acid levels, including highly toxic secondary bile acids, resulting in fewer sensitive bacteria and more bile-tolerant bacteria. This may explain the fact of reducing Prevotella (Bacteroidetes), which is known as sensitive to bile acids. In particular, the higher F / B ratio is associated with obesity and is always consistent.⁴⁴ Recent studies have shown that modified intestinal microbiota with increased F / B ratio promotes obesity caused by diet by influencing bile acid profile modulating host metabolism via X intestinal farnesoid receptor. A study on Firmicutes found two species related to fat, namely Oscillibacter sp and Erysipelotrichaceae sp. and some Clostridiales species associated with fat indirectly. The high F / B microbial community comprising these species may speed up obesity associated with modern diets. For example, bacteria such as Oscillibacter are known as intestinal microbes that manage intestinal dysfunction caused by high fat [45].

Antibiotic in early life

Repeated exposure to antibiotics early in life, especially β -lactam agents, is associated with increased weight and height. Previous studies have found that the use of antibiotics as early

as age is associated with increased risk of obesity in childhood [46]. It is found that antibiotic exposure in the second or third trimester of pregnancy may be associated with higher risk of childhood obesity, and higher central adiposity and percent body fat. The existence of different human microbiological placenta and maternal exchange of commensal microbiota may occur before birth, through the exchange of placental bacteria, will breed intestinal microbes in the fetus. With intake of antibiotics during pregnancy, which can cause intestinal microbiology across the placenta and enter the fetal circulation, may interfere with the normal colonization of the intestinal microbial growth in the uterus similar to the effect after the birth. In short, children born with antibiotic intake in the second or third trimester of pregnancy are at high risk for obesity in childhood.

This exposure is independent of each potential mixer and others, including mothers with BMI, birth weight and breastfeeding mother. If other observational studies can imitate findings on antibiotics, pregnancies and childhood obesity, then the logical next step to determine whether this association is the possible causes for follow-up children whose mothers take part in a controlled antibiotic test randomly during pregnancy. Prospective observation studies of conception through childhood, with the measurement of a series of microbial communities, body weight and body composition, and a complete set of factors that trigger potential are required. Better understanding of how delivery and early antibiotics affect microbiotics can pave the way for newborns to prevent obesity and related illness.

Breastfeeding Practices

Breast feeding increase Bifidobacteriaceae (Actinobacteria phylum) and Enterobacteriaceae proteobacteria phylum) and decrease Lachnospiraceae (firmicutes), Veillonellaceae (firmicutes), and Ruminococcaceae (firmicutes). Exclusive breastfeeding is shown beneficial while introduction with early formula milk alter gut dysbiosis [47]. In this study, the relationship between the composition of infant milk microbiotics at six points during the first two years of life and development of obesity in childhood. Intestinal microbiota for the first 2 years of life, especially at the age of two, is strongly associated with small BMI. Interestingly, the BM-z score at the age of two is not much higher in children who then become overweight or obesity, so the development of intestinal microbiota composition predicts then BMI is preceded by any weight that can be measured in children. Intestinal microbiota is also associated with infant growth and overweight or obesity rates. This suggests that microbiota infants are associated with BMI of childhood and overweight or obese mothers. In addition, the taxonomies in intestinal microbiota that are most related to these features show a great overlap with the infant taxonomy most relevant to the BMI of childhood. It is possible that the intestinal microbiota found to be associated with BMI then reflects the environmental aspects, particularly nutrition and lifestyle, which may have a direct risk of obesity. Diet is one of several factors that affect intestinal microbiota and intestinal microbiota can mediate obesity caused by diet. The relationship between intestinal microbiota in 1 and 2 years and later the BMI of childhood can be partly due to dietary factors precursors of obesity and

increasingly forming the intestinal microbiota of the baby at that age.

Maternal/Pregnancy Related

Mode of delivery sequential mediator pathway involving birth mode and Firmicutes species richness of microbiota of the infant gut, namely, higher abundance of Lachnospiraceae for the intergenerational transmission of overweight [48]. Besides that, both maternal weight status and caesarean delivery shape early-life gut microbial development and the weight outcome of offspring, for which a mediation role for gut microbiota has been posited.

Genetic

Salivary Amylase gene was long associated with obesity in human. Gut microbiota analyses revealed a positive correlation between AMY1 copy number and Prevotella abundance.36 Prevotella were found to be protective of obesity in many studies. Possible natural adaptation of the body system where gut microbiota colony is regulated by specific gene. Adult and child intestinal microbiome with high AMY1 copy numbers have been enriched in the Prevotella genus and species, but not with any generic microbiota intestine. Prevotella is one of the largest enterotypes in intestinal microbiomes and has enzymes and gene clusters essential to the fermentation and use of complex polysaccharides. This is consistent with the results of some studies showing Prevotella increasingly metabolic, and the high Prevotella / Bacteroides ratio supports weight loss in response to dietary intervention. However, there is significant associations of the high AMY1 CN with both normal weight loss and Prevotella abundance (especially Prevotella copri), the abundance of Prevotella is not significantly associated with nutritional status or with the amount of dietary carbohydrates (including fibre and starch).

Environment Home Disinfectant (daily)

Exposure to household disinfectants was associated with higher BMI at age 3, mediated by gut microbial composition at age 3-4 months [49]. Although child overweight was less common in households that cleaned with eco-friendly products, the lack of mediation by infant gut microbiota suggests another pathway for this association. The use of home cleaning products affects intestinal microbiota at 3-4 months of age, baby exposure to antibiotics, birth modes, breastfeeding and other covariance altering microbes. The connection with the modified microbiota is the most appealing to the home use of dehydration, which shows the lack of the genus Haemophilus and the genus Clostridium. This change is compatible with the killings of disinfection bacteria containing bleach and hydrogen peroxide. At the same time, Lachnospiraceae is 1.3 times more likely to be given to the baby's intestinal microbiota after frequent cleansing with disinfection. This enrichment with Lachnospiraceae at 3-4 months of age is highly predictive of BMI z score at the age of 1 which in turn is a strong determinant of BMI z score at age 3. Additionally, evidence of statistical mediation by Lachnospiraceae fecal association between weekly cleaning up to a day with disinfectant during the baby

and increased BMI z score at 3 years of age.

Fecal fortune of Lachnospiraceae baby increases with disinfectant cleaning frequency in a dose-dependent way, with 2 times higher probability of higher microbial abundance in the highest use category. Usually found in infants with traceable Lachnospiraceae, 31 genus Ruminococcus being 1.6 times more likely to have higher efficacy with disinfectant disinfection in the study baby. The genus Haemophilus is further reduced when disinfecting germs are used daily, in line with their extraordinary sensitivity to the high concentration of hydrogen peroxide and the use of frequent household removal products.

Associations between the use of cleansing products and changes in infant intestine microorganisms are dependent on dose and show consistency with the shaft and murine model experiments, meet 2 Bradford Hill additional criteria for causation. Finally, the test for mediation contributes evidence of the association of microbiological changes to the development.

Limitation

The large number of species of gut microbiome make species specific association with childhood obesity is difficult. Besides, different technologies or measurement tools were used across studies to present the abundance of the gut microbiota. Therefore, it is not possible to make pool estimation on the risk factors due to the heterogeneity of the outcome measured.

Future recommendation

Knowledge on specific groups or species is beneficial in order to design a specific intervention for childhood obesity prevention. Besides, the Bifidobacterium and Lactobacillus is recommended to be used as a healthy indicator of obesity among obese children and adolescents, as well as a range of prebiotic supplements and probiotics in the diet for additional supplements for obesity. Knowing the factors that contribute to obesity and obesity problems can be used as a predictive tool for prevention purposes. Apart from that, a dose response relationship also can be further analyzed between the association of the gut microbiome with obesity.

CONCLUSION

The dynamic changes of microbiomes composition were associated with adiposity among children since birth. Bacteroidetes phylum should be further researched for its potential protective mechanism. Besides, further understanding on the factors effecting gut microbiome will improve the preventive effort to combat childhood obesity. Additional studies are needed to determine the mechanisms that may lead to increased adiposity in this population and further research could help to guide what populations could benefit from the protective effects of probiotics against obesity.

Conflict of Interest

None declared.

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Table 1: Summary of Included Studies

Author, year	Study design	Location	Sample	Risk factors/effect	Measurement	Finding	NOS
Bai et al. 2019 ²⁵	Cohort study	America	N= 267 Aged 7 – 18 years	- BMI Exercise frequency diet - controlling for age, sex and use of antibiotics and probiotics	-BMI -Gut microbiota	-significant associations were found between the gut microbiota composition and BMI level and lifestyles controlling for demographic and clinical factors in children. -gut microbiota of 7–18-year-old children was dominated by the bacterial phyla Firmicutes, Bacteroidetes -Proteobacteria, like findings in preadolescents and adolescents	7
Dogra S et al 2015 ³⁹	Cohort study	Singapore	N= 75 infants	-Composition of bacteria -Delivery mode -Gestation duration	Adiposity at 18 months	- (Firmicutes phylum) levels are positively correlated with the difference in skinfold thickness -High Bifidobacterium and Collinsella at a later age had lower adiposity at 18 months of age	6
Forbes JD et al. 2018 ⁴⁷	Cohort Study	Canada	1087 infants	Breastfeeding (EBF)	-weight for length exceeding the 85th percentile gut microbiota features at 3 to 4 months	-EBF increase Bifidobacteriaceae (Actinobacteria phylum) and Enterobacteriaceae proteobacteria phylum) -EBF decrease Lachnospiraceae (firmicutes), Veillonellaceae (firmicutes), and Ruminococcaceae (firmicutes). Increase Lachnospiraceae subsequently overweight, risk overweight,	7
Hollister et al. 2018 ³³	Non-Randomized trial	America	N= 55 (2-5 years old)	-Weight reduction	BMI	-Bacteroides massiliensis was significantly enriched in obese children, while B. plebius was significantly enriched in NW controls during weight reduction process. -significant correlations between multiple Bacteroides-like OTUs and BMI z-score, -neither F:B ratios nor OTU-level abundances were altered in conjunction with weight change in the obese children.	Poor Quality*
Hou et al. 2017 ³⁵	Cohort study	China	N=87 (3-18 years)	-Weight reduction	16S rRNA gene, the enterotypes, and quantity of the gut microbiota	-Bifidobacterium and Lactobacillus increased among the obese children during the process of weight reduction. -Bifidobacterium and Lactobacillus might be used as indicators of healthy conditions among obese children.	4
Goffredo M et al. 2016 ²⁶	Cohort study	America	N=84 (12-18yrs)	-Firmicutes to Bacteroidetes ratio -abundance of Bacteroidetes and Actinobacteria	body mass index, visceral and SC fat (all $P < .05$)	relationship of the gut microbiota and short chain fatty acids with obesity and fat partitioning	5
Karlsson Videhult F et al. 2015 ¹⁹	double-blind, randomized, placebo-controlled intervention trial,	Sweden	N=179 (4-30 months, follow up until 8-9 yrs. old)	Probiotic Lactobacillus paracasei ssp. paracasei F19 (LF19)	BMI	-Feeding LF19 during infancy did not modulate body composition, growth or any of the assessed metabolic markers at school age. -The steady increase in probiotic products targeting infants and children calls for long-term follow-up of initiated probiotic intervention studies.	Fair quality*
Karvonen et al. 2019 ³¹	Cross-sectional study	Boston, Us	N=502 3-year-old	-Maternal pre-pregnancy BMI - birth weight and length, -formula feeding during the first year, --high frequency of fast food consumption, -time watching TV or computer screen at 3 years.	16 SrRNA sequencing (V4 region) of the stool samples were performed with Illumina MiSeq.	Some of the differences in gut composition of bacteria between obese and non-obese adults can already be observed in 3-year old children.	6
León-Mimila P et al. 2018 ⁵⁰	Case-Control Studies	Mexico	N=921 (6-12 years)	salivary amylase gene (AMY1)	Anthropometry AMY1 CNV Prevotella abundance	gut microbiota analyses revealed a positive correlation between AMY1 copy number and Prevotella abundance MY1 CNV was significantly associated with obesity Role of genetic in regulating gut microbiota	5
López-Contreras BE et al.2018 ⁵¹	Cross sectional	Mexico	N=138 6–12 years.	Insoluble fibre intake	Anthropometric Dietary record 16S rNA	-Bacteroides eggertii abundance was significantly higher in obese children and correlated positively with body fat percentage and negatively with insoluble fibre intake. -suggests that increased fibre intake reduces the abundance of Lipopolysaccharide-synthesizing bacteria, preventing metabolic endotoxemia and consequently obesity-	8

Author, year	Study design	Location	Sample	Risk factors/effect	Measurement	Finding	NOS
						associated complications, such as insulin resistance	
Maya-Lucas et al. 2019 ⁵²	Cohort Study	Mexico	N=20 children (9 and 11 years)	Bacteroidetes Firmicutes	metagenomic shotgun sequencing metabolic profile	The gut microbiome of normal and obese children, was quite similar in their microbial communities or their protein assemblies. However, individual members of the gut microbial communities, which are significantly different between normal and obese children, affect the synthesis of some metabolites via specific routes, promoting obesity in addition to other genetic and environmental factors to which the children are exposed.	7
Mbakwa et al. 2016 ⁴⁶	Cohort study	Netherland	N=979 children (full term at birth followed up until 8 yr.)	Antibiotic	Anthropometric outcomes (age- and sex standardized body mass index, weight and height z-scores, and overweight) were measured repeatedly at 7 time	Repeated exposure to antibiotics early in life, especially b-lactam agents, is associated with increased weight and height. Specific antibiotic used was not associated with body mass index z-scores and overweight.	7
Mbakwa et al. 2018 ⁵³	Cohort	Netherland	N=295 (6 to 7 years)	Gut microbiota	age- -sex -BMI -weight z scores	Identified bacterial groups were found to be associated with overweight.	6
Mousavi et al. 2018 ²⁷	Cross-sectional study	Iran	188 children (7 - 12 years)	Gut microbiota, firmicutes and Bacteroidetes CFU, and so bact/firm ratio	Anthropometry Real time pcr international physical activity questionnaire (IPAQ),	The amount of Bacteroidetes and so bact/firm ratio were decreased among obese children; however; Firmicutes increased. It was suggested that obesity in children might be associated with the imbalance of gut microbes.	7
H.M. Tun et al. 2018 ⁴⁸	Cohort	Canada	N=935 full-term infants	-Birth mode -prepregnancy overweight	Maternal overweight Caesarean section	Both maternal weight status and caesarean delivery shape early-life gut microbial development and the weight outcome of offspring, for which a mediation role for gut microbiota has been posited. novel sequential mediator pathway involving birth mode and Firmicutes species richness of microbiota of the infant gut, namely, higher abundance of Lachnospiraceae for the intergenerational transmission of OWOB	4
Nicolucci et al. 2017 ⁵⁴	a single-centre, double-blind, placebo-controlled trail	Calgary, Canada	7-12 Intervention = 22, control = 19	Prebiotic (BMI)	Mean different of BMI	In a placebo-controlled, randomized trial, we found a prebiotic (OI) to selectively alter the intestinal microbiota and significantly reduce body weight z-score, percent body fat, percent trunk fat, and serum level of interleukin 6 in children with overweight or obesity	Good quality*
Nirmalkar et al. 2018 ³⁰	Cross sectional	Mexico	111 6-11 yrs.	Firmicutes Bacteroides ³⁰³⁰³⁰³⁰	16s rDNA	The characterization of gut bacterial diversity by high-throughput DNA sequencing of V3-16S rDNA libraries showed higher relative abundance of Firmicutes and lower relative abundance of Bacteroidetes in obese children	6
Orbe-Orihuela et al. 2018 ³⁷	Cross-sectional	Mexico	890 6-14 years old	Bacteroidetes Firmicutes	Anthropometric features and blood biochemical parameters Real-Time PCR (qPCR) Cytokines measurement in blood	A remarkable finding in our study was the presence of inflammation markers associated with microbiota and childhood obesity. We found a positive correlation of TNF-α serum concentrations, with BMI adjusted by age and dependent on the microbiota profile. A correlation of 16% of TNF-α levels was observed in those children with the highest tertile of Firmicutes RA and 13% with the lowest tertile of Bacteroidetes Relative Abundance	5
Riva et al. 2017 ²⁹	Cross Sectional	Italy	6-16 YRS 78	Bacteroidetes* (MEAN SD)	BMI z-score Short chain fatty acids Microbiota composition (16s rNA gene) microbiota composition	associated with an altered gut microbiota characterized by elevated levels of Firmicutes and depleted levels of Bacteroidetes. Our results suggest that gut microbiota dysbiosis and elevated fermentation activity may be involved in the aetiology of childhood obesity	6
Stanislawski et al. 2018 ⁵⁵	Cohort	Norway	N=165 Newborn	Breastfeeding Maternal pregnancy weigh gain	Maternal pregnancy weigh gain. Questionnaire on breast feeding Child BMI PCR for bacterial quantification	Association between the infant gut microbiota and later BMI, and they offer preliminary evidence that the infant gut microbiota, particularly at 2 years of age, may have potential to help identify children at risk for obesity.	4
HM Tun et al. 2018 ⁴⁹	Cohort	Canada	N=757 infants	Use of disinfectants	Questionnaire on home environment and personal use of cleaning products	Exposure to household disinfectants was associated with higher BMI at age 3, mediated by gut microbial composition at age 3-4 months. Although child overweight was less common in households that cleaned with eco-friendly	6

Author, year	Study design	Location	Sample	Risk factors/effect	Measurement	Finding	NOS
						products, the lack of mediation by infant gut microbiota suggests another pathway for this association	
Zhong et al. 2019 ³⁶	Self-controlled clinical trial	shanghai	N=38 (3-16 yrs.)	Genetic factors prone to obesity dietary intervention rich in non-digestible carbohydrate	-bacteria enterotypes	children genetically obese with Prader-Willi syndrome shared a similar dysbiosis in their gut microbiota with those having diet-related obesity. A diet rich in non-digestible but fermentable carbohydrates significantly promoted beneficial groups of bacteria and reduced toxin-producers, which contributes to the alleviation of metabolic deteriorations in obesity regardless of the primary driving forces.	Poor quality*
Nakayama et al. 2017 ³²	Cross sectional	Philippines	N=43 7-9 yrs.	High fat Western diet	-antibiotic administration -dietary questionnaire -The high-throughput 16S rRNA gene sequence analysis	Overweight and obese children living in Ormoc, who consumed a high-fat diet, harboured microbiota with higher F/B ratio and low abundance of <i>Prevotella</i> . The altered gut microbiota may be a sign of a modern diet-associated obesity among children in developing areas.	5

Table 2: Factors Affecting Gut Microbiota in Children

Categories	Factors	Effect to Microbiota	Microbiota measures	Association	Effect to obesity	Authors
Diet	High fat diet	Decrease	Provetella (Bacteroidetes)	Wilcoxon RS (p=0.023)	Increase	Zhong 2019 ³⁶
	Vegetarian/Omnivore	Decrease	Actinobacteria	(p=0.01)	Reduce	Bai et al 2019 ²⁵
	Insoluble Fibre intake	Decrease	B. Eggerti	r=-0.307	Reduce	Lopez CBE 2018 ⁵¹
Breastfeeding among infants	Breastfeeding less than 6 months vs exclusive BF	Increase	Lachnospiraceae, F/B ratio	OR 1.98 (1.22-3.20), OR 2.02 (1.25-3.27)	Increase	Forbes et al. 2018 ⁴⁷
	Formula milk only	Increase	Lachnospiraceae F/B ratio	OR 1.47 (0.87-2.45) *, OR 1.77 (1.07-2.91)	Increase	Forbes et al. 2018 ⁴⁷
	Breast feeding + formula feeding at 3 months	Increase	Lachnospiraceae F/B ratio	OR 1.37 (0.90-2.09) *, OR 1.52 (1.00-2.32)	Increase	Forbes et al. 2018 ⁴⁷
Maternal/Pregnancy related	Antimicrobial during pregnancy	Increase	Firmicutes	r=0.4145, p=0.0001	Increase	Riva et al. 2017 ²⁸
		Decrease	Bacteroidetes	r= -0.4538, p<0.0001	Increase	Riva et al. 2017 ²⁸
	Mode of delivery born vaginally	Increase	Bacteroides	OR 3.33 (1.49-7.41)	Reduce	H. M. Tun et al. 2018 ³⁸
	Mode of delivery cesarean-delivered + maternal OWOB (joint effect)	Increase	Lachnospiraceae	aOR, 2.02; 95% CI, 1.06-3.87	Increase	H. M. Tun et al. 2018 ³⁸
	Obesity/weight gain during pregnancy	Increase	Bifidobacterium bifidum	(p= 0.32)	Increase	Stanislawski et al. 2018 ⁵⁵
Environment /others	Home Disinfectant (daily)	Increase	Lachnospiraceae	aOR 1.93(1.08,3.45)	Increase	H. M. Tun et al. 2018 ⁴⁹
		Increase	Ruminococcus	aOR 1.55(1.10,2.17)	Increase	H. M. Tun et al. 2018 ⁴⁹
		Increase	Coriobacteraceae	aOR 1.47(1.05,2.06)	Increase	H. M. Tun et al. 2018 ⁴⁹
	Physical activity	Decrease	F/B ratio	r=0.129 p=0.77	Reduce	Mousavi et al. 2018 ²⁷
	Antibiotic in early life	Decrease	Bacteroidetes	aOR 0.89 (0.67 to 1.19)	Increase	Mbakwa et al. 2016 ⁴⁶
	Antibiotic	Decrease	Bacteroidetes	aOR 0.24; 95% CI 0.03-0.44)	Increase	Mbakwa et al. 2016 ⁴⁶

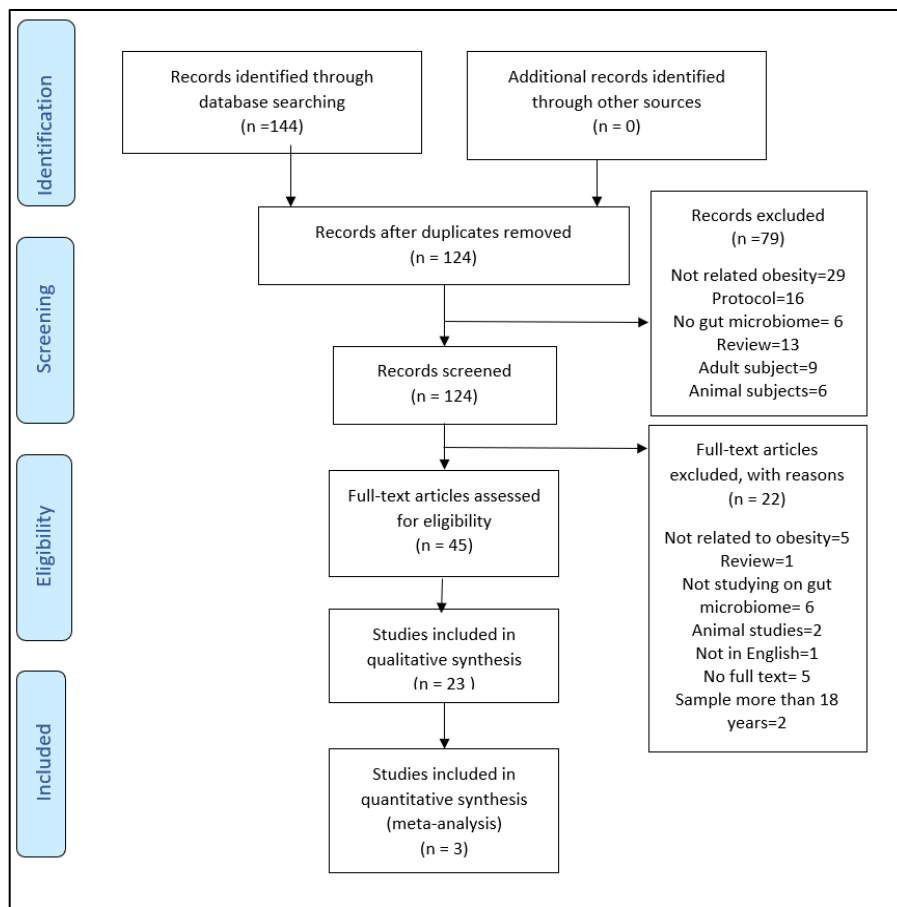


Figure 1: Process of Study Selection

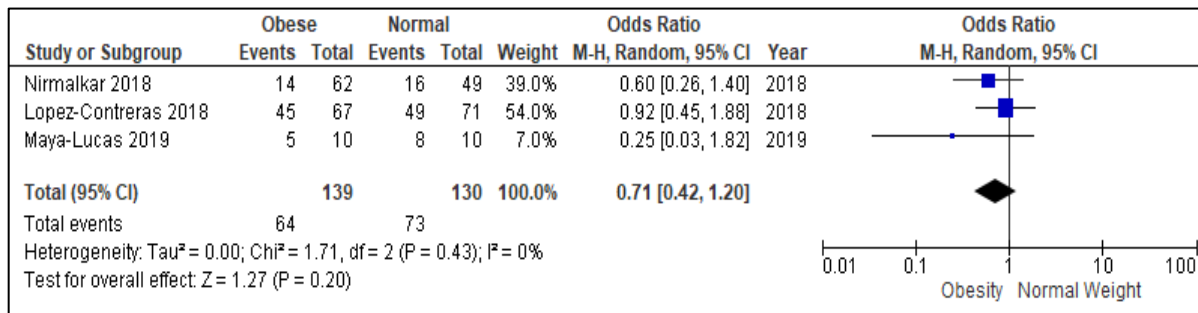


Figure 2: Forest Plot: Presence of Bacteroidetes as Predominant taxa

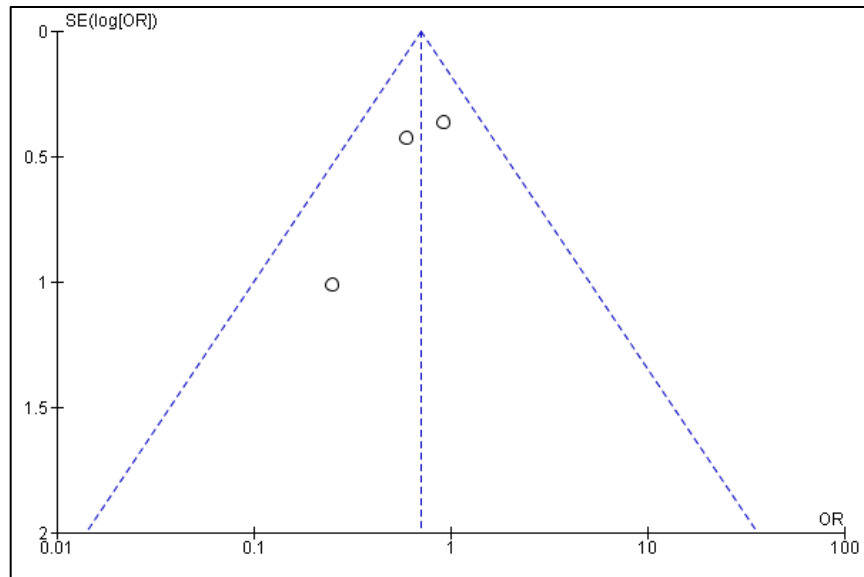


Figure 3: Funnel plot for publication bias