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GUT HEALTH DIGEST

UNIVERSITY OF WASHINGTON STRATEGIC ANALYSIS, RESEARCH & TRAINING (START) CENTER

REPORT TO THE BILL & MELINDA GATES FOUNDATION

NOVEMBER 1, 2017

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3. **Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infant.** {Abstract & START editorial comment} {Full article}
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    - Stool samples of 15 infants collected at varying time points during the first month of life are analyzed for bacterial and viral composition as well as similarity to the mother's microbiota.

8. **Fecal microbiota analysis of children with small intestinal bacterial overgrowth among residents of an urban slum in Brazil.** {Abstract & START editorial comment} {Full article}
    - Cross-sectional study evaluating the relationship between SIBO and fecal microbiota composition and SES factors.

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10. **Non-digestible oligosaccharides directly regulate host kinome to modulate host inflammatory responses without alterations in the gut microbiota.** {Abstract & START editorial comment} {Full article}
    - Systems biology approach is used to investigate the signaling responses of intestinal epithelial cells exposed to two different prebiotics.

DETAILS OF ARTICLES

1. Natural polyreactive IgA antibodies coat the intestinal microbiota
   Bunker J, Erickson S, Flynn T, Henry C, Koval J, Meisel M, et al.
   Science. 358(6361). 2017 October 20.
   PubMed ID: 28971969

ABSTRACT
Large quantities of immunoglobulin A (IgA) are constitutively secreted by intestinal plasma cells to coat and contain the commensal microbiota, yet the specificity of these antibodies remains elusive. Here we profiled the reactivities of single murine IgA plasma cells by cloning and characterizing large numbers of monoclonal antibodies. IgAs were not specific to individual bacterial taxa but rather polyreactive, with broad reactivity to a diverse, but defined, subset of microbiota. These antibodies arose at low frequencies among naïve B cells and were selected into the IgA repertoire upon recirculation in Peyer's patches. This selection process occurred independent of microbiota or dietary antigens. Furthermore, although some IgAs acquired somatic mutations, these did not substantially influence their reactivity. These findings reveal an endogenous mechanism driving homeostatic production of polyreactive IgAs with innate specificity to microbiota.

WEB: 10.1126/science.aan6619
IMPACT FACTOR: 37.205
CITED HALF-LIFE: >10.0

START EDITORIAL COMMENT: While it is widely agreed that immunoglobulin A (IgA) play an important role in the microbiota, their mechanism of action is debated. Some suggest IgA are highly specific for a single component of the microbiota to which they bind with high-affinity, others suggest that they are polyreactive, and can bind to several different components in the microbiota with low-affinity. The authors attempted to explore these relationships and distinguish microbiota-reactivity from a broad pattern of polyreactivity by assessing the frequency of polyreactive specificities using a panel of seven common and structurally diverse antigens to screen monoclonal antibodies (mABs) from naïve B cells and IgA repertoires. Enzyme-linked immunosorbent assay (ELISA) was used for this screening, which included the following seven antigens: DNA, insulin, lipopolysaccharide, flagellin, albumin, cardiolipin, and keyhole-limpet hemocyanin. Figure 1 outlines the microbiota-reactivity and polyreactivity of IgA plasma cell (PC) populations from the small intestine (SI), colon, bone marrow, salivary gland and Lactating Mammary Gland, as well as several populations of naïve B cell subsets. Figure 1B and 1C demonstrates that SI IgA mAbs had much higher levels of microbiota reactivity than splenic B2 cells. The investigators concluded that their observations suggest that all B cell populations demonstrate microbiota-reactive specificities, but such specificities are enhanced in IgA subsets originating from the small intestine. Microbiota-reactive and polyreactive specificities accounted for 83% of SI-derived IgA versus only 45% of the naïve B-cell population. Figure 1D further supports the investigator's conclusions that IgA mAbs are more polyreactive than other B cells, as it shows the percentage of mAbs from each cell line that had the given number of positive reactivities (with the highest being 7).

2. Effect of probiotics on perinatal outcome in patients at high risk of preterm birth.
   Kirihara N. Kamoitomo M, Tabira T, Hasimoto T, Taniguchi H, Maeda T.
   Journal of Obstetrics and Gynecology Research. 2017 October 10. [epub ahead of print]
   PubMed ID: 28994162

ABSTRACT
Aim: Recent reports have shown lower levels of *Clostridium* and higher levels of *Lactobacillales* in the intestinal microbiota in preterm birth patients compared to term birth patients. However, the influence of probiotics on perinatal status has not been elucidated. The aim of our study was to evaluate the effects of probiotics on perinatal outcomes.

Methods: We retrospectively evaluated the effects of oral probiotics on perinatal outcome in patients at high risk of preterm birth. Probiotics containing *Streptococcus faecalis*, *Clostridium butyricum* and *Bacillus mesentericus* were administered for prophylaxis of bacterial vaginosis or treatment of constipation starting at 12.5 ± 4.1 weeks until delivery. Patients not administered probiotics were defined as the non-probiotics group. Between these two groups, perinatal outcomes including gestational age at birth, birth weight, chorioamnionitis or funisitis and preterm birth before 32 weeks were compared. In addition, multivariate regression analyses were performed to evaluate factors influencing preterm birth before 32 weeks, chorioamnionitis/funisitis and normal vaginal flora.

Results: The probiotics group showed longer gestation, higher birth weight, lower rates of chorioamnionitis and higher rates of normal vaginal flora compared to the non-probiotics group. Multivariate regression analysis showed that probiotics significantly suppressed preterm birth before 32 weeks and tended to suppress chorioamnionitis/funisitis. The adjusted odds ratios (95% confidence interval) for preterm birth before 32 weeks and chorioamnionitis/funisitis were 0.05 (0.01–0.71) and 0.07 (0.01–1.03), respectively.

Conclusions: Oral probiotics containing Clostridium had a significant effect on the prevention of preterm birth before 32 weeks of gestation.

WEB: 10.1111/jog.13497
IMPACT FACTOR: 5.574
CITED HALF-LIFE: 5.3

START EDITORIAL COMMENT: The investigators retrospectively analyzed preterm birth outcomes in 121 patients classified as high-risk pregnancies. 45 women took probiotics (PB group) during pregnancy and 76 had not (non-PB group). All women in the PB group were given tablets containing *Streptococcus faecalis, Clostridium butyricum* and *Bacillus mesentericus.* Table 2 shows the different perinatal outcomes in each group, highlighting that in addition to fewer preterm births before 32 weeks, women in the PB group had a significantly higher vaginal flora of *Lactobacillus*. The authors point out that this observation supports previous findings by Wilks *et al*. that showed the presence of *Lactobacillus* in the vagina was associated with reduced risk of Choriamnionitis (CAM) and consequently a lower rate of preterm births. Table 5 shows a univariate and multiple regression analysis of factors that affect *Lactobacillus* presence in the vaginal flora. Probiotic use was the only significant factor. The authors hypothesized that the probiotics tested operate to increase the duration of gestation by the following mechanisms: (i) increasing intestinal *Clostridium* (ii) increasing the T cells in the intestines and cervix, (iii) suppressing hyperimmunization against infection and (iv) suppressing the change in cervical status. Collectively these mechanisms were thought to increase the maternal host tolerance of the fetus, which

resulted in fewer preterm births. Some important limitations of the study include its retrospective design that prevented the measurement of important potential confounders, the lack of intestinal microbiota measurement in patients, and the lack of randomization. That is, an obstetrician provided probiotics based on their judgment that a patient was at increased risk for bacterial vaginosis or needed treatment for constipation (prophylaxis vs. treatment).

3. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infant.
   Paganini D, Uyoga M, Kortman G, Cercamondi C, Moretti D, Jaeggi T, et al.
   Gut microbiota. 66(11). 2017 November
   PubMed ID: 28774885

ABSTRACT

OBJECTIVE: Iron-containing micronutrient powders (MNPs) reduce anaemia in African infants, but the current high iron dose (12.5 mg/day) may decrease gut *Bifidobacteriaceae* and *Lactobacillaceae*, and increase enteropathogens, diarrhoea and respiratory tract infections (RTIs). We evaluated the efficacy and safety of a new MNP formula with prebiotic galacto-oligosaccharides (GOS) combined with a low dose (5 mg/day) of highly bioavailable iron.

DESIGN: In a 4-month, controlled, double-blind trial, we randomised Kenyan infants aged 6.5-9.5 months (n=155) to receive daily (1) a MNP without iron (control); (2) the identical MNP but with 5 mg iron (2.5 mg as sodium iron ethylenediaminetetraacetate and 2.5 mg as ferrous fumarate) (Fe group); or (3) the identical MNP as the Fe group but with 7.5 g GOS (FeGOS group).

RESULTS: Anaemia decreased by ≈50% in the Fe and FeGOS groups ($p<0.001$). Compared with the control or FeGOS group, in the Fe group there were (1) lower abundances of Bifidobacterium and Lactobacillus and higher abundances of Clostridiales ($p<0.01$); (2) higher abundances of virulence and toxin genes (VTGs) of pathogens ($p<0.01$); (3) higher plasma intestinal fatty acid-binding protein (a biomarker of enterocyte damage) ($p<0.05$); and (4) a higher incidence of treated RTIs ($p<0.05$). In contrast, there were no significant differences in these variables comparing the control and FeGOS groups, with the exception that the abundance of VTGs of all pathogens was significantly lower in the FeGOS group compared with the control and Fe groups ($p<0.01$).

CONCLUSION: A MNP containing a low dose of highly bioavailable iron reduces anaemia, and the addition of GOS mitigates most of the adverse effects of iron on the gut microbiome and morbidity in African infants.

WEB: 10.1136/gutjnl-2017-314418
IMPACT FACTOR: 16.658
CITED HALF-LIFE: 8.7

START EDITORIAL COMMENT: Galacto-oligosaccharide (GOS) is a prebiotic that selectively promotes the growth of *Lactobacillus* and *Bifodobacterium* species in the gut. These two species support gut integrity and *Bifidobacterium* can sequester iron (Fe), reducing its availability to pathogens that require iron for growth. These two beneficial bacterial species are often reduced following administration of high levels of Fe, which may be a factor in previous observations of increases in the number of enteropathogenic *E.coli*, local inflammation and diarrhea incidence following administration of micronutrient powder (MNP) with Fe formulations. The authors hypothesized that administering GOS in conjunction with MNP and a bioavailable Fe formulation would restore gut integrity and reduce the number of adverse events associated with increased iron intake. Figures 3 and 4 show the differences in gut microbial composition at 3 weeks and 4 months after starting the interventions. These figures show that gut microbiota diversity is greatly reduced in children receiving MNPs containing Fe supplementation alone. The diversity is restored and closely resembles that of the MNP control group in children who received MNP+Fe with GOS. The investigators conclude that the addition of GOS to a formulation of MNP+Fe

counterbalances the bacteria growth-limiting effect of the Fe by increasing the levels of *Bifodacterium* and *Lactobacillus* and maintaining a diverse gut microbiota. In line with these conclusions, children who received MNP+Fe+GOS or MNP alone had a significant reduction in the number of respiratory tract infections (RTIs) treated at Month 4 compared to those who received MNP+Fe (Figure 6). Plasma I-FABP, a marker for enterocyte injury, was also significantly higher in the MNP+Fe group than the control, while no significant difference was seen between the MNP+Fe+GOS group and control. These observations suggest that GOS helps maintain a normal gut microbiota composition in the presence of Fe administration, which reduces enterocyte damage and limits the growth of harmful bacteria which carry virulence factors known to cause disease. Addition of GOS to the MNP+Fe is an effective way to ensure that children receive and metabolize adequate iron to reduce anemia and sequester excess iron in the gut that alter microbiota composition and increase disease.

4. Does the maternal vaginal microbiota play a role in seeding the microbiota of neonatal gut and nose?
   Sakwinska O, Foata F, Berger B, Brüssow H, Combremont S, Mercenier A, et al.
   Beneficial Microbes. 8(5): 763-778. 2017 October 13.
   PubMed ID: 29022384

ABSTRACT
The acquisition and early maturation of infant microbiota is not well understood despite its likely influence on later health. We investigated the contribution of the maternal microbiota to the microbiota of infant gut and nose in the context of mode of delivery and feeding. Using 16S rRNA sequencing and specific qPCR, we profiled microbiota of 42 mother-infant pairs from the GUSTO birth cohort, at body sites including maternal vagina, rectum and skin; and infant stool and nose. In our study, overlap between maternal vaginal microbiota and infant faecal microbiota was minimal, while the similarity between maternal rectal microbiota and infant microbiota was more pronounced. However, an infant's nasal and gut microbiota were no more similar to that of its own mother, than to that of unrelated mothers. These findings were independent of delivery mode. We conclude that the transfer of maternal vaginal microbes play a minor role in seeding infant stool microbiota. Transfer of maternal rectal microbiota could play a larger role in seeding infant stool microbiota, but approaches other than the generally used analyses of community similarity measures are likely to be needed to quantify bacterial transmission. We confirmed the clear difference between microbiota of infants born by Caesarean section compared to vaginally delivered infants and the impact of feeding mode on infant gut microbiota. Only vaginally delivered, fully breastfed infants had gut microbiota dominated by *Bifidobacteria.* Our data suggest that reduced transfer of maternal vaginal microbial is not the main mechanism underlying the differential infant microbiota composition associated with Caesarean delivery. The sources of a large proportion of infant microbiota could not be identified in maternal microbiota, and the sources of seeding of infant gut and nasal microbiota remain to be elucidated.

WEB: 10.3920/BM2017.0064
IMPACT FACTOR: 2.923
CITED HALF-LIFE: 2.7

START EDITORIAL COMMENT: This study was performed on mother-infant pairs from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort. All infants were of normal gestational age, and born via Caesarean section (n=8) or vaginal delivery (n=34). Investigators examined microbiota composition over time and between different groups of children, and compared microbiota between mothers and infants. Figure 1 shows the results of the microbiota analyses from samples collected at different body sites in the mothers and infants. Figure 1B highlights that the composition of the microbiota in infant nasal and stool samples changed drastically between birth and week 3 of life, with *Bifodobacterium* dominating in the gut, and *Staphylococcus aureus* in the nose. Figure 2 shows the differences in microbiota in day 3 infant stool between vaginally delivered breastfed (V-B), vaginally delivered mixed fed (V-M) and Caesarean delivered mixed-fed (C-M). In breastfed babies, *Bifidobacterium* dominated the microbiota composition, while mixed feeding increased the abundance of *Klebsiella, Escherichia* and *Streptococcus mitis.* The proportion of *Bacteroides* remained unchanged between breastfed and mixed-fed children delivered vaginally, but this genus was not present at all in the Caesarean group. Figures 3 and 4 show comparisons of the similarity between the infant microbiota and the mother's microbiota and different sites and time-points. Very little similarity was seen in the populations, questioning the previous theory that the infant microbiota is seeded by the mother. Additionally, the authors suggest that the differences seen in microbiota between vaginally delivered

infants and Caesarean delivered infants is more influenced by the differences in feeding practices in these groups, and not by the lack of exposure to the vaginal flora in the Caesarean group. However, the study is limited by its small sample size and low number of exclusively breastfed babies. This study is also limited by the fact that their sample contained just two infants who were exclusively formula-fed, and no exclusively breast fed babies in the Cesarean group, so some feeding comparisons could not be made.

5. Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation
   Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, et al.
   Science. 358(6361):359-365. 2017 October 20.
   PubMed ID: 29051379

ABSTRACT
Intestinal colonization by bacteria of oral origin has been correlated with several negative health outcomes, including inflammatory bowel disease. However, a causal role of oral bacteria ectopically colonizing the intestine remains unclear. Using gnotobiotic techniques, we show that strains of *Klebsiella spp.* isolated from the salivary microbiota are strong inducers of T helper 1 (TH1) cells when they colonize in the gut. These *Klebsiella* strains are resistant to multiple antibiotics, tend to colonize when the intestinal microbiota is dysbiotic, and elicit a severe gut inflammation in the context of a genetically susceptible host. Our findings suggest that the oral cavity may serve as a reservoir for potential intestinal pathobionts that can exacerbate intestinal disease.

WEB: 10.1126/science.aan4526
IMPACT FACTOR: 37.205
CITED HALF-LIFE: >10.0

START EDITORIAL COMMENT: The investigators used several different techniques to analyze the relationship between oral and gut microbiota, with respect to immune response in the gut. The study suggests that oral *Klebsiella* can multiply in the gut and induce a strong T_H1 response. Investigators transplanted saliva samples from two patients with Crohn's disease (CD) into germ-free (GF) mice and examined their immune responses after six weeks. This experiment showed that the immune response in mice from one of the patients had markedly elevated interferon-γ^+ $CD4^+$ T cells (T_H1). The researchers then aerobically cultured cecal contents in these mice and examined the bacterial colonies present through 16*S* rRNA sequencing, where eight different predominant species were identified. These strains were cultured and reintroduced into GF mice to measure T_H1 response. As can be seen in Figure 1, when the *Klebsilla* strain, Kp-2H7, was administered by itself, it induced a strong T_H1 response that was not seen when a mixture of the other seven strains were administered (Mix-7). In an attempt to understand the mechanism in which *Klebsilla* induces a T_H1 response in the gut, the investigators ran several experiments with the strain in wild-type and knock out mice, or between mice administered an array of different antibiotics (in drinking water) and those who were not. The results from these experiments can be seen in Figure 3, and suggest that antibiotic exposure may enable orally derived Kp-2H7 colonization by disrupting the colonization resistance provided by specific members of the gut microbiota that are sensitive to ampicillin and tylosin, but resistant to metronidazole and spectinomycin. Finally, the investigators confirmed their findings by inoculating the saliva from two additional healthy patients and two patients with ulcerative colitis (UC) into GF mice. As seen with the CD patient, the saliva from one of the UC patients induced a high T_H1 response in the mouse colon. Cecal strains from these mice were cultured and the same experiments were run to confirm the findings.

6. Childhood stunting in relation to the pre- and postnatal environment during the first 2 years of life: The MAL-ED longitudinal birth cohort study
   MAL-ED Network Investigators.
   PLoS Medicine. 14(10):e1002408. 2017 October 25.
   PubMed ID: 29069076

ABSTRACT
Background: Stunting is the most prevalent manifestation of childhood malnutrition. To characterize factors that contribute to stunting in resource-poor settings, we studied a priori selected biological and social factors collected longitudinally in a cohort of newborns.

Methods and findings: We enrolled 1,868 children across 7 resource-poor settings in Bangladesh, Brazil, India, Nepal, Peru, South Africa, and Tanzania shortly after birth and followed them for 24 months between 2 November 2009 and 28 February 2014. We collected longitudinal anthropometry, sociodemographic factors, maternal-reported illnesses, and antibiotic use; child feeding practices; dietary intake starting at 9 months; and longitudinal blood, urine, and stool samples to investigate non-diarrheal enteropathogens, micronutrients, gut inflammation and permeability, and systemic inflammation. We categorized length-for-age Z-scores into 3 groups (not stunted, ≥−1; at risk, <−1 to −2; and stunted, <−2), and used multivariable ordinal logistic regression to model the cumulative odds of being in a lower length-for-age category (at risk or stunted). A total of 1,197 children with complete longitudinal data were available for analysis. The prevalence of having a length-for-age Z-score below −1 increased from 43% (range 37%–47% across sites) shortly after birth (mean 7.7 days post-delivery, range 0 to 17 days) to 74% (16%–96%) at 24 months. The prevalence of stunting increased 3-fold during this same time period. Factors that contributed to the odds of being in a lower length-for-age category at 24 months were lower enrollment weight-for-age (interquartile cumulative odds ratio = 1.82, 95% CI 1.49–2.23), shorter maternal height (2.38, 1.89–3.01), higher number of enteropathogens in non-diarrheal stools (1.36, 1.07–1.73), lower socioeconomic status (1.75, 1.20–2.55), and lower percent of energy from protein (1.39, 1.13–1.72). Site-specific analyses suggest that reported associations were similar across settings. While loss to follow-up and missing data are inevitable, some study sites had greater loss to follow-up and more missing data than others, which may limit the generalizability of the findings.

Conclusions: Neonatal and maternal factors were early determinants of lower length-for-age, and their contribution remained important throughout the first 24 months of life, whereas the average number of enteropathogens in non-diarrheal stools, socioeconomic status, and dietary intake became increasingly important contributors by 24 months relative to neonatal and maternal factors.

WEB: 10.1371/journal.pmed.1002408
IMPACT FACTOR: 11.862
CITED HALF-LIFE: 7.1

START EDITORIAL COMMENT: The investigators evaluated 22 child and maternal exposures (such as enteropathogen detection in non-diarrheal stool, markers of gut inflammation, markers of gut permeability, and percent of dietary calories from protein) for their relationship with linear growth faltering in the first two years of life. Interestingly, there were several child exposures that were not associated with being in a lower length-for-age category across all time points, including gut inflammation (neopterin and myeloperoxidase), gut permeability (lactulose:mannitol ratio), micronutrient status and days of exclusive breastfeeding. However, as seen in Figure 6, the association between AAT (a marker of barrier disruption) and AGP (a marker of of acute-phase systemic

inflammation) vary by age, with greater impact at older ages. The authors hypothesize that this suggests gut and systemic inflammation may protect against growth faltering at early ages, but not as a child grows. Figures 4 and 5 show the interquartile cumulative odds ratios of being in a lower length-for-age category obtained from the multivariable ordinal logistic regression model for five contributing factors (enrollment weight-for-age, maternal height, WAMI index, % energy from protein, and enteropathogen detection) overall and by site. Figure 4A highlights that, with the exception of enrollment-weight-for-age, the contribution of the variables to the odds of being stunted increased as the children age. Figure 5 shows that the contributions of these factors to stunting varied by country, with the most variation seen in the % energy from protein. This factor is a stronger predictor of a low z-score in Bangladesh, India, and Tanzania, which are considered to be some of the poorest sites in the study. The authors note this variability in study sites as a limitation of the study, as well as the necessity of omitting all data from Pakistan (due to errors in height measurements). In addition, the Brazil study site had high loss to follow-up rates, which may help explain why linear growth data from this site do not follow the trends observed elsewhere. Finally, in this study, the analyses considered total number of enteropathogens present rather than specific pathogens of interest. Prior studies have suggested that only a subset of enteropathogens appear to affect growth, therefore additional insights could be gained if the authors compared enteropathogen composition.

7. Evolution in fecal bacterial/viral composition in infants of two central African countries (Gabon and Republic of the Congo) during their first month of life.
   Brazier L, Elguero E, Koumavor C, Renaud N, Prugnolle F, Thomas F, et al.
   PLoS One. 12(10): e0185569. 2 October 2017
   PubMed ID: 28968427

ABSTRACT
Few studies have analyzed the gut microbiota of child in unindustrialized countries, but none during the first month of life. Stool samples were collected from healthy newborns in hospitals of Gabon (n = 6) and Republic of the Congo (n = 9) at different time points during the first month of life: meconium, day 2 (D02), day 7 (D07) and day 28 (D28). In addition, one fecal sample was collected from each mother after delivery. Metagenomic sequencing was performed to determine the bacterial communities, and multiplex real-time PCR was used to detect the presence of seven enteric viruses (rotavirus a, adenovirus, norovirus I and II, sapovirus, astrovirus, enterovirus) in these samples. Bacterial diversity was high in the first days of life, and was dominated by the genus *Prevotella*. Then, it rapidly decreased and remained low up to D28 when the gut flora was composed almost exclusively of strictly anaerobic bacteria. Each infant's fecal bacterial microbiota composition was significantly closer to that of their mother than to that of any other woman in the mothers' group, suggesting an intrauterine, placental or amniotic fluid origin of such bacteria. Moreover, bacterial communities differed according to the delivery mode. Overall, the bacterial microbiota communities displayed a similar diversification and expansion in newborns within and between countries during the first four weeks of life. Moreover, six of the fifteen infants of this study harbored enteric viruses (rotavirus, enterovirus and adenovirus) in fecal samples, but never in the meconium. A maternal source for the viruses detected at D02 and D07 can be excluded because none of them was found also in the child's mother. These findings improve our knowledge on the gut bacterial and viral communities of infants from two Sub-Saharan countries during their first month of life.

WEB: 10.1371/journal.pone.0185569
IMPACT FACTOR: 2.806
CITED HALF-LIFE: 3.7

START EDITORIAL COMMENT: The investigators looked at the bacterial and viral composition of the gut microbiome in 15 different infants from Gabon and the Republic of the Congo. Among these, there was a mixture of different combinations of delivery modes, gestational age at birth and feeding type, all variables which are thought to affect the microbiota. There were no viral infections seen in the meconimum (first stool), but at least one enteric virus was found in 15 children in the proceeding 28 days. These viruses were not found in the samples of the mother. There were no statistical associations between virus detection and country, birth modes, feeding modes or abundance of bacterial genera. Figure 3 shows the relative abundance of aerobic and anaerobic bacteria among the infants and mothers enrolled. With some exceptions, strict anaerobes did not predominate among these infants prior to the day 28 stool assessment. In terms of bacterial diversity (Shannon index) and generic richness, there were no significant differences according to delivery mode, country, or feeding method. However, analysis of the composition of bacteria found in samples from Cesarean and vaginally delivered infants differed. *Bacteroides* and *Collinsella* predominated in vaginally delivered infants and *Sarcina* and *Klebsiella* in those delivered via C-section. Analysis of the microbiota of maternal-infant pairs showed infants shared a greater number of OTUs with their mother than with another mother in the study chosen at random (Table 3). The maternal-infant microbiota closeness (similarity) increased by day 28. This result is different from what was observed in the Sakwinska study highlighted in this digest,

in which they found no difference in statistical closeness between infant-mother pairs compared to other women in the study. The techniques used in each study to compare closeness were different, and both had a relatively low number of samples, so further exploration of this relationship is needed. The current study showed that there is high bacterial diversity in the meconimum, which declines rapidly after birth, and rebounds by day 28. This finding suggests that bacterial colonization starts before birth, contrary to common belief that the uterus is a sterile environment.

8. Fecal microbiota analysis of children with small intestinal bacterial overgrowth among residents of an urban slum in Brazil.
   Mello C, Rodrigues M, Filho H, Melli L, Tahan S, Pignatari A, Morais M.
   Jornal de Pediatria. 16 October 2017 [ePub ahead of print].
   PubMed ID: 29049893

ABSTRACT
Objective: To analyze the fecal microbiota composition of children living in an urban slum in Brazil, with or without small intestinal bacterial overgrowth, and to investigate the occurrence of stunting and anemia.

Methods: A total of 100 children were studied, aged 5–11 years, from the municipality of Osasco, São Paulo. Small intestinal bacterial overgrowth was screened through hydrogen and methane breath test with lactulose. Weight and height were measured, and the height-for-age and body mass-for-age anthropometric indexes were calculated. The occurrence of anemia was investigated by capillary hemoglobin. Analysis of bacterial phylum, genus, and species was performed by real-time polymerase chain reaction in fecal samples.

Results: Small intestinal bacterial overgrowth (SIBO) was identified in 61.0% of the children. A lower mean of height-for-age Z-score ([−0.48 ± 0.90] vs. [−0.11 ± 0.97]; p = 0.027), as well as capillary hemoglobin ([12.61 ± 1.03 g/dL] vs. [13.44 ± 1.19 g/dL]; p < 0.001) was demonstrated in children with SIBO when compared with children without small intestinal bacterial overgrowth. Children with small intestinal bacterial overgrowth presented a higher frequency of Salmonella spp., when compared to those without small intestinal bacterial overgrowth (37.7% vs. 10.3%; p = 0.002). Higher counts of total Eubacteria (p = 0.014) and Firmicutes (p = 0.038) were observed in children without small intestinal bacterial overgrowth; however, a higher count of Salmonella (p = 0.002) was found in children with small intestinal bacterial overgrowth.

***Conclusion*:** Children who lived in a slum and were diagnosed with small intestinal bacterial overgrowth showed lower H/A Z-scores and hemoglobin levels. Furthermore, differences were observed in the fecal microbiota of children with small intestinal bacterial overgrowth, when compared to those without it; specifically, a higher frequency and count of Salmonella, and lower counts of Firmicutes and total Eubacteria.

WEB: 10.1016/j.jped.2017.09.003
IMPACT FACTOR: 2.081
CITED HALF-LIFE: 6.9

START EDITORIAL COMMENT: This study sought to ascertain a link between SIBO and nutrition status in children living in an urban slum in Brazil, and examined children's gut microbiome composition. In order to be included in the study, children had to have an absence of diarrhea and absence of antibiotic use in the previous month. Similar to a previous study of SIBO among children in Bangladesh, this study observed lower height for age z-scores among children with SIBO (as determined by glucose hydrogen breath test) compared to those without (Table 1). Table 3 presents the phyla, genera, and species of bacteria composing the fecal microbiota of children with or without SIBO. Children with SIBO had significantly higher levels of *Salmonella* spp. than those without SIBO. The authors note that because children recruited into the study did not actively have diarrhea or GI symptoms that are characteristic of pathogenic *Salmonella* infections, this finding indicates there are a high number of asymptomatic

infections in this population. In addition, SIBO was significantly associated with a lack of running water in the child's household. Authors suggest that health care practitioners might consider testing children living in households without running water for SIBO, particularly if they demonstrate signs of growth faltering. A major limitation of this study is the use of real-time polymerase chain reaction for examination of the gut microbiome composition, as this method only allows the assessment of pre-selected bacterial groups.

9. Prebiotics Mediate Microbial Interactions in a Consortium of the Infant Gut Microbiome.
   Medina D, Pinto F, Ovalle A, Thomson P, Garrido D.
   International Journal of Molecular Sciences. 18(10): 2095. 4 October 2017.
   PubMed ID: 28976925

ABSTRACT
Composition of the gut microbiome is influenced by diet. Milk or formula oligosaccharides act as prebiotics, bioactives that promote the growth of beneficial gut microbes. The influence of prebiotics on microbial interactions is not well understood. Here we investigated the transformation of prebiotics by a consortium of four representative species of the infant gut microbiome, and how their interactions changed with dietary substrates. First, we optimized a culture medium resembling certain infant gut parameters. A consortium containing *Bifidobacterium longum* subsp. *infantis, Bacteroides vulgatus*, *Escherichia coli* and *Lactobacillus acidophilus* was grown on fructooligosaccharides (FOS) or 2'-fucosyllactose (2FL) in mono- or co-culture. While *Bi. infantis* and *Ba. vulgatus* dominated growth on 2FL, their combined growth was reduced. Besides, interaction coefficients indicated strong competition, especially on FOS. While FOS was rapidly consumed by the consortium, *B. infantis* was the only microbe displaying significant consumption of 2FL. Acid production by the consortium resembled the metabolism of microorganisms dominating growth in each substrate. Finally, the consortium was tested in a bioreactor, observing similar predominance but more pronounced acid production and substrate consumption. This study indicates that the chemical nature of prebiotics modulate microbial interactions in a consortium of infant gut species.

WEB: 10.3390/ijms18102095
IMPACT FACTOR: 3.226
CITED HALF-LIFE: 3.3

START EDITORIAL COMMENT: Figure 7 shows the results of exposing the four bacteria consortium to 2FL, which is found in human milk oligosaccharides (Fig 7A-C), or FOS, which is found in most infant formulas (Fig 7D-E), in a validation bioreactor. Interestingly, both substrates were entirely consumed by the bacteria, however, there was both lactate and acetate production in the 2FL-exposed group, while the FOS-exposed group showed mostly lactate production. Consistent with this observation, *B. infantis* dominated the co-culture exposed to 2FL after 24 hours, while *L. acidophilus* dominated the FOS co-culture. These results parallel the differences in bacterial composition seen in stool samples from breastfed and formula-fed infants, and provide a mechanistic understanding of why certain bacteria thrive under each feeding mode.

10. Non-digestible oligosaccharides directly regulate host kinome to modulate host inflammatory responses without alterations in the gut microbiota.
    Wu R, Määttänen P. Napper S, Scruten E, Li B, Koike Y, et al.
    Microbiome. 5(1): 135. 10 October 2017.
    PubMed ID: 29017607

ABSTRACT
BACKGROUND: Prebiotics are non-digestible food ingredients that enhance the growth of certain microbes within the gut microbiota. Prebiotic consumption generates immune-modulatory effects that are traditionally thought to reflect microbial interactions within the gut. However, recent evidence suggests they may also impart direct microbe-independent effects on the host, though the mechanisms of which are currently unclear.

METHODS: Kinome arrays were used to profile the host intestinal signaling responses to prebiotic exposures in the absence of microbes. Identified pathways were functionally validated in Caco-2Bbe1 intestinal cell line and in vivo model of murine endotoxemia.

RESULTS: We found that prebiotics directly regulate host mucosal signaling to alter response to bacterial infection. Intestinal epithelial cells (IECs) exposed to prebiotics are hyporesponsive to pathogen-induced mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) activations, and have a kinome profile distinct from non-treated cells pertaining to multiple innate immune signaling pathways. Consistent with this finding, mice orally gavaged with prebiotics showed dampened inflammatory response to lipopolysaccharide (LPS) without alterations in the gut microbiota.

CONCLUSIONS: These findings provide molecular mechanisms of direct host-prebiotic interactions to support prebiotics as potent modulators of host inflammation.

WEB: 10.1186/s40168-017-0357-4
IMPACT FACTOR: 8.496
CITED HALF-LIFE: 2.4

START EDITORIAL COMMENT: The investigators compared the host kinome response between cell cultures exposed to two prebiotics, inulin or scFOS, both before and after challenge by an enterohemorrhagic *E. coli* (EHEC) serotype. In-vitro experiments with intestinal epithelial cells showed there were 79 shared differentially phosphorylated peptides (DPP) between the two treatments in the absence of EHEC, but only 32 shared DPPs post-challenge. Further analysis of the pathways affected by the different DPP profiles showed that inulin most affected pathways related to response to stimuli and biological regulation, while scFOS affected pathways for metabolic processes, cellular processes and immune system processes. Figure 2 shows the pathways and protein kinases most affected by each prebiotic, supporting the investigators' conclusion that prebiotics are strong modulators of the host immune response. The expression of certain inflammatory cytokines and chemokines differed between the pre-biotic supplemented mice groups and those given sham saline solution (PBS) when challenged with LPS (Figure 5). Prebiotic supplemented and non-supplemented mice did not exhibit systematic differences in microbial diversity or in the relative abundance of the top 5 clustered orthologous group functional categories for colonic microbiota (Figure 6). Therefore, in the absence of microbes, the prebiotics tested were able to directly regulate the phosphorylation of select host signaling molecules to alter mucosal responses to injury (following EHEC challenge (in-vitro) or LPS challenge (in-vivo)). Interestingly, while both prebiotics tested directly inhibited NF-κB and MAPK signaling, only scFOS

inhibited phosphorylation of p38 MAPK, suggesting that each prebiotic has a distinct mechanism by which they affect the immune system pathways.

ADDITIONAL ARTICLES OF INTEREST

A Gut Microbial Mimic that Hijacks Diabetogenic Autoreactivity to Suppress Colitis

Correlates of multi-drug non-susceptibility in enteric bacteria isolated from Kenyan children with acute diarrhea.

Identification of Enteric Viruses in Oral Swabs from Children with Acute Gastroenteritis.

The effect of fiber and prebiotics on children's gastrointestinal disorders and microbiome.

Preterm Infant-Associated Clostridium tertium, Clostridium cadaveris, and Clostridium paraputrificum Strains: Genomic and Evolutionary Insights.

Prebiotic inulin-type fructans induce specific changes in the human gut microbiota.

Genome sequencing of 39 Akkermansia muciniphila isolates reveals its population structure, genomic and functional diverisity, and global distribution in mammalian gut microbiotas.