Whites Science
A Scientific Erudition

THE CHARACTERISTICS OF BACTERIAL GROWTH IN THE STOOLS OF NEWBORNS IN THE FIRST DAYS OF LIFE

***A.Riskin[1], A.Kugelman[1], I.Srugo[2], M.Hemo[1], A.Toropine[1], D.Bader[1]**
[1]Department of Neonatology[1] and Microbiology Laboratory[2], Bnai Zion Medical Center, Rappaport Faculty of Medicine, Technion, Haifa, Israel
[2]Bnai Zion Medical Center, 47 Golomb St., Haifa 31048, Israel

ABSTRACT

Background: Neonatal gut colonization starts immediately after birth. It is affected by mode of delivery, type of infant's feeds and antibiotics use.

Objective: To identify the bacteria cultured in the stools of term newborn infants, and characterize associations to mode of delivery and type of infant's feeds.

Methods: 32 newborns (birth-weight 3351±616 g, gestational age 39.2±1.4 weeks) were included. 43 stool cultures collected on day of life 1.3±0.6. In 11 (34.4%) newborns there was no growth, in 18 (56.2%) E.Coli grew, and only in 3 (9.4%) there was growth of Lactobacilli. Of the 5 infants fed breast milk only: 2 had no growth, 2 grew Lactobacilli and 1 – E.Coli. Of the 8 fed formula only: 4 had no growth, 1 grew Lactobacilli and 3 had E.Coli. Of the 19 fed on breast milk and formula: 5 had no growth and 14 grew E.Coli (Chi sqiare=10.53 ,p=0.032). In a multi-variate model breast milk feeding only (as opposed to formula or mixed feedings) was associated with increased chance of no growth or lactobacillus growth in feces (OR=10.74; 95% CI 0.92-125.68 ,p=0.040); cesarean delivery (as opposed to vaginal) concomitantly increased the chance for no growth or Lactobacillus (OR=5.09; 95% CI 0.96-25.90, p=0.052). Combined model of human milk and cesarean delivery was significant (p=0.027).

Conclusions: Human milk feeding after delivery inhibits normal fecal flora growth and encourages probiotic bacterial growth. Cesarean delivery without passage through the birth canal might contribute to this.

Key words: stools; newborns; Lactobacilli, E.Coli; breast milk; mode of delivery.

INTRODUCTION

Gut colonization starts immediately after delivery and usually takes few days (1-4). Probiotic bacteria support gut defense mechanisms and strengthen host defense against pathogenic infections (3). Mode of delivery (passage through the birth canal as opposed to cesarean section), infant's nutrition (human milk versus formula), developing as opposed to developed countries, and perinatal and neonatal antibiotic treatment were mentioned among the factors that influence the nature of gut microbial growth in the newborn (2-6).

Our aim in this study was to identify the bacteria in the stools of healthy newborns after delivery, their time of colonization of the gut, and its relation to mode of delivery and the treatment given to the newborn after delivery.

Corresponding author:
Email: arik.riskin@gmail.com

MATERIALS AND METHODS

The study was conducted in the newborn nursery of Bnai Zion Medical Center and was approved by its IRB. Stool samples were collected on a daily basis from newborns in the department. Data regarding the newborns' gender, birth weight, gestational age, mode of delivery (vaginal, assisted or cesarean), nutrition

(mother's milk only, formula or mixed), and whether the mother or the infant were given antibiotics pre- or post-partum, was collected from the mothers and infants' medical records.

Stool samples were collected in clean dry containers and were immediately sent to the microbiology lab. 0.05 g (50 mcg) of the fresh stool specimens were immediately put into tubes with 5 cc normal saline (NaCL0.9%) and diluted 10 times with normal saline (10^{-2} to 10^{-8}). 0.05 cc of each of these dilutions were incubated on different media in order to isolate the different types of bacteria (Enterobacteria-MacConkey agar; Gram-positive aerobic bacteria-phenyl-ethyl alcohol blood agar; Anaerobic bacteria-blood-agar containing amikacin; Lactic acid bacteria-rogosa agar). In order to isolate anaerobic bacteria cultured agars were incubated in 37ºC for 5 days, while for aerobic bacteria they were cultured in 37ºC for 1-2 days. Identification of Lactobacilli and anaerobic bacteria was done with Api-rapid 32 A (Bio Merie ux. France). Isolation of aerobic bacteria was done by regular methods.

Data was statistically analyzed using descriptive statistics, Student's t-test, ANOVA for parametric or Kruskal-Wallis on medians for non parametric data, Chi-square, General Linear Model (GLM) for linear regression (with calculation of Least Square Means and use of Bonferroni method for multiple comparisons), binary logistic regression, logistic multiple regression, stepwise forward procedure for multi-variate regression and mediation analysis (SigmaStat version 2.03, Chicago, IL; Minitab version 12.23, State College, PA; SAS version 9.2, Cary, NC, USA). Results are presented as means ± standard deviations (and medians if relevant) and Odds Ratio (OR) and 95% Confidence Interval (CI). P-value of less than 0.05 was considered as statistically significant.

RESULTS

The study included 32 newborns (18 males and 14 females) (1.3:1.0) that were born with a mean birth-weight of 3351±616 g after 39.2±1.4 weeks of gestation. Altogether 43 stool samples were collected on 1.3±0.6 days of life. In 11 (34.4%) of the infants no growth was identified at an average of 1.9±0.8 (median 2.0, range: 1-3) days of age at the time of stool sampling. In 21 (65.6%) growth was first noted on 2.8±2.7 (median: 2.0, range: 1-4) days of life. In 18 of them growth of E.Coli was noted on 2.3±0.7 (median: 2.0, range: 1-4) days of life, and only 3 had growth of Lactobacilli on 6.0±6.9 (median: 2.0, range: 2-14) days of life (Kruskal-Wallis test on medians: p=0.38, not significant (NS)).

The following associations with factors that could affect the time and nature of bacterial stool growth were examined:

A. Mode of delivery: Of the 32 newborns included in this study, 21 (65.6%) were born vaginally and 11 (34.4%) were delivered by cesarean section. Of those delivered vaginally: 6 (28.5%) had no growth, 1 (4.8%) grew Lactobacillus and 14 (66.7%) grew E.Coli. Of those delivered by cesarean section: 5 (45.4%) had no growth, 2 (18.2%) grew Lactobacilli and 4 (36.4%) grew E.Coli (Chi sqiare=3.16, p=0.21, NS).

B. Antibiotic pre-and peri-partum treatment given to the mother (usually for prolonged rupture of membranes of more than 18 hours or other risk factors for infection): Of 5 (15.6%) such mothers, 1 (20%) infant had no growth, and 4 (80%) grew E.Coli in their stools (Chi sqiare=1.51, p=0.47, NS).

C. Post-partum antibiotic treatment given to the newborn (for a combination of two or more peri-partum risk factors for infection): Only one (3.1%) such infant, who grew E.Coli in stools (Chi sqiare=0.80, p=0.67, NS).

D. Infants' feedings: 5 (15.6%) were fed on human (own mother's) milk only, 8 (25%) were fed formula only, and 19 had mixed feedings (mother's milk and formula). Of the 5 given mother's milk only 2 (40%) had no growth, 2 (40%) grew Lactobacilli and only one (20%) grew E.Coli. Of the 8 who were fed on formula only: 4 (50%) had no growth, 1 (12.5%) grew Lactobacillus and 3 (37.5%) grew E.Coli. Of the 19 infants who got mixed feedings: 5 (26.3%) had no growth and 14 (73.7%) grew E.Coli (Chi sqiare=10.53, p=0.032, significant)

To assess the combination of factors that could affect stool bacterial growth we used multi-variate regression models. Since very few had growth of Lactobacillus (possibly because

more days are required for its growth, see discussion), we have used a model that compared no growth or probiotic bacterial growth (in our case only Lactobacillus) (14 infants, 43.8%) vs. potentially pathogenic bacterial growth (in our case E.coli) (18 infants, 56.2%). For nutrition we have compared those newborns on mother's milk only (5 infants, 15.6%) vs. all the infants who either got formula only or mixed feedings) (27 infants, 84.4%). Preliminary statistical analyses of various combinations of factors have shown that these combinations gave the most informative possibility to try and identify the effects on stool bacterial growth.

Uni-variate analyses have shown that the odds (OR=Odds Ratio) and 95%CI (Confidence Interval) for no growth or positive Lactobacillus in stools of newborns were as follows:

A. **Mode of delivery:** cesarean section (without passage through the birth canal) vs. vaginal delivery increased the chance for no growth or Lactobacillus in stools by 3.5 (OR=3.50; 95% CI 0.76-16.12 ,p=0.100).

B. **Antibiotics** given to the mother before delivery increased the odds for no growth or positive Lactobacillus in stools by 0.27 (OR=0.27; 95% CI 0.03-2.73, p=0.226).

C. **Antibiotics** given to the infant after delivery decreased the chance for no growth or Lactobacillus in stools by 1.31 (OR=1.31; 95% CI 0.07-22.93, p=0.885).

D. **Feeding own mother's milk only** increased the odds for no growth or only positive Lactobacillus growth in stools of newborns by 6.8 (OR=6.80; 95% CI 0.66-69.64, p=0.071).

Thus, in the multi-variate model we have included only human milk (from mother) vs. formula or mixed feedings, and mode of delivery (cesarean vs. vaginal) (p-values ≤ 0.10), and found that they augmented each others effect. In the mutual model mother's milk nutrition increased the odds for no growth or Lactobacillus in stools by 10.74 (OR=10.74; 95% CI 0.92-125.68, p=0.040, significant), and cesarean delivery increased the odds for no growth or Lactobacillus in stools by 5.09 (OR=5.09; 95% CI 0.96-25.90, p=0.052, borderline significant). Although both 95%CI's are still borderline for the independent variables, the combined model where we included mother's milk only nutrition and cesarean delivery (without passage through the birth canal) was statistically significant (p=0.027).

DISCUSSION

In this study we have shown that bacterial gut colonization of newborns was mainly affected by their nutrition (7). Nutrition that is based only on mother's milk increases the odds for Lactobacillus (which is a probiotic bacteria) growth in the stools, or at least for no growth of other (potentially pathogenic) bacteria in the time frame we have examined stool cultures of newborns after delivery. Human milk and breastfeeding encourage colonization of Lactobacilli in the newborn's gut (7-9). Cesarean section (without passage in the birth canal, as opposed to regular vaginal or assisted deliveries) increased the odds for no growth or Lactobacillus growth in the newborns' stools. Previous studies have shown that gut colonization is delayed in newborns delivered by cesarean sections, but colonization with Bifidobacteria (another type of probiotic bacteria) was also delayed in these newborns (6, 7, 10, 11). As opposed to this, nutrition that was not based on mother's milk only (mixed or formula) and vaginal delivery were found to be associated with earlier growth of normal gut flora (E.Coli in our case), which potentially could become pathogenic. The serotypes of E.Coli that grew in infants stools after vaginal deliveries were similar to those grown from their mothers' stools, which raise the possibility of infection by maternal bacteria around the birth canal (7). Another association between cesarean delivery and our findings is possible, because initiation of breastfeeding could be delayed after cesarean delivery because of maternal recovery from the surgery. In this case cesarean delivery could mediate the delay in breastfeeding. We have tried to test such a model statistically, but the results were negative (data not shown), possibly because our sample was relatively small and thus under-powered to check this.

Our study limitations are first and above all related to our sample size which was relatively small (32 newborns, 43 stool samples), thus making it very hard to reach statistical

significance, especially regarding the different effects on gut colonization. Another limitation was our method that was based on stool cultures, which are not sensitive enough, especially for growing probiotic bacteria – we have grown only Lactobacillus and not other bacteria, such as Bifidobacteria (7), which is a very important probiotic bacterium that dominates gut flora of breastfeeding infants by the age of one week. Molecular biology methods are by far more sensitive in identification of these bacteria and should be preferred in future studies. Another limitation was the relatively short time we had for collecting stools for culture, which was dictated by the relatively short hospitalizations of most infants in the newborn nursery after delivery. Bacteria, and especially probiotic bacteria, grow later (in one of the cases we had it grown after 14 days in an infant who was born by cesarean delivery and whose mother was re-admitted to the hospital with him). This is one of the reasons why we have decided in our models to include the newborns without growth along with those who grew Lactobacilli in their stools. It may be claimed that such model could be problematic and these two conditions would not necessarily go together, especially in cesarean deliveries.

In conclusion, feeding newborns after delivery with mothers' milk only would delay the growth of normal gut flora and encourage colonization of the gut by probiotic bacteria. Mode of delivery (lack of passage through the birth canal) could contribute to this. Future studies should preferably be based on genetic identification of bacteria in the stools using molecular biology techniques that are far more sensitive than stool cultures.

REFERENCES

1. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; 361:512-9.

2. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. *Acta Paediatr Suppl* 2003;91:48-55.

3. Salminen S, Isolauri E. Intestinal colonization, microbiota, and probiotics. *J Pediatr* 2006;149:S115-20.

4. Caicedo RA, Schanler RJ, Li N, Neu J. The developing intestinal ecosystem: implications for the neonate. *Pediatr Res* 2005;58:625-8.

5. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breastfed infants. Acta Paediatr Suppl 1999;88:47-57.

6. Biasucci G, Benenati B, Morelli L, et al. Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J Nutr* 2008: 138: 1796S–800S.

7. Morelli L. Postnatal development of intestinal microflora as influenced by infant Nutrition. *J Nutr*. 2008; 138: 1791S-1795S.

8. Martı´n R, Langa S, Reviriego C, et al. Human milk is a source of lactic acid bacteria for the infant gut. *J Pediatr*. 2003;143:754–8.

9. Civardi E, Garofoli F, Mazzucchelli I, et al. Enteral nutrition and infections: the role of human milk. *Early Hum Dev* 2014;90:S57-9.

10. Gronlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr*. 1999;28:19–25.

11. Azad MB, Konya T, MaughanH, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013;185:385-94.