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Intestinal colonization in Polish infants: From newborns till 18-month-old children

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ABSTRACT

Introduction: Human intestinal colonization is a dynamic process that is nowadays redefining due to hygienic changes in the Polish population.

Aim: To analyze the development of the intestinal flora from newborns till 18-month-old infants in Poland.

Material and methods: 171 newborns were enrolled. We collected fecal samples at 5 timepoints (1st stool, at 3, 6, 12, 18 months). At each visit, the questionnaire concerning breastfeeding, antibiotics, probiotics was obtained including atopy family history at the first visit.

Results: The count of staphylococci, enterococci, lactobacilli decreased (mean 0 months vs. 18 months: 3.08×10^7 CFU/g vs. 6.35×10^6 CFU/g; 1.85×10^{10} CFU/g vs. 9.26×10^7 CFU/g; 3.3×10^{11} CFU/g vs. 3.11×10^7 CFU/g) and *Clostridium difficile* and Gram-negative bacilli increased (6.2×10^4 CFU/g vs. 1.34×10^5 CFU/g; 1.78×10^6 CFU/g vs. 9.03×10^7 CFU/g) during the first 18 months of life. Positive maternal atopy history influenced colonization with staphylococci in newborns, anaerobic bacteria, enterococci in 3-month-old infants and anaerobic bacteria in 6-month-old infants.

Discussion: Our study shows that the gut colonization is a constant process. For the first time, we present the trends in bacterial establishment in a group of more than 170 Polish children.

The positive role of breastfeeding in the establishment of gut flora was previously suggested. Unexpectedly, among mostly breastfed children no relation between breastfeeding and the infantile gut microflora was found.

Conclusions: The intestinal colonization is continuously changed over the first 18 months of life and is influenced by positive maternal atopy history.

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1. Introduction

The human adult gastrointestinal microflora is, under normal circumstances, a stable ecosystem. The opposite situation is observed during the postnatal period, when intestinal microflora is a subject for constant changes.

For over 20 years scientists were trying to follow up its establishment and the factors influencing its composition in early infancy.[1,2] The background for these studies was that bacteria in human gut may induce long-term consequences for the child's health[3] and a single pathogen may become a source of infection, especially in preterm infants.[4]

Bacteria colonize the intestinal tract immediately after delivery, firstly predominated by *Escherichia coli* and enterococci and later enriched with anaerobic bacteria such as bifidobacteria, clostridia and *Bacteroides*.[5] Other anaerobic bacteria are successively acquired, creating a highly diverse microflora found in older children.[6] Yet, the process of colonization is complicated and it might be influenced by a bunch of different factors as the hospital environment during the delivery, prematurity, hygiene habits in the population, type of infants feeding,[7] antibiotics application or probiotics substitution.[8] The environmental sources seem to be very important because the differences in the colonization pattern were previously shown between developing and western societies e.g. Ethiopian–Swedish newborns[9] or Estonian and Swedish infants.[10]

2. Aim

The present study aimed to characterize the composition of fecal flora in the country that used to be encountered as developing country and in the last decade its population changed the nutritional and living habits, similarly to Western societies. We additionally assessed the role of breastfeeding and positive atopy family history in the establishment of intestinal flora in newborns and infants.

3. Material and methods

3.1. Patients

During a 3-year period at the Department of Pediatrics, Medical University of Silesia, 171 healthy newborns born vaginally were recruited to the present study. All enrolled newborns were discharged from the hospital at standard time after 72 h. In total, 120 18-month-old children finished the study. We have not obtained full 5 fecal samples from 51 infants due to inconsistent parental appearance during follow up. The inclusion criteria were: healthy, born at term newborns without any signs of infection (neither maternal infection or child's infection at perinatal period) and uncomplicated pregnancy. The parents were informed verbally and in writing regarding the nature and requirements of the study. Their written informed consent was obtained, and the study was approved by the Ethical Committee of Medical University of Silesia.

3.2. Clinical evaluation

During each visit the children were clinically examined by the same pediatrician and the questionnaire was filled out including information about family size, household, type of feeding, antibiotic treatment and oral probiotic supplementation. The study was designed to provide 4 pediatrician's visits (at age 3, 6, 12 and 18 months) with fecal samples collection. The enrollment in our study included muster of the first newborn's feces for microbiologic analysis and establishment of atopy family history for each newborn.

3.3. Fecal bacterial analysis

Approximately 1 g voided stool was collected into sterile plastic containers by the parents (1st stool after delivery was taken by qualified nurse at the delivery room) at the day of pediatrician's visit. All samples were kept at −20°C for not more than 3 months.

All fecal samples were cultured on Schaedler Agar, sequentially diluted (from 10^{-2} to 10^{-9}) and inoculated on selective media (BioMerieux, Warsaw, PL): yeasts and fungi on Sabouraud medium with chloramphenicol as described above; aerobic bacteria were cultured on MacConkey agar; Chapman medium and D-Coccosel agar.

Anaerobic bacteria were incubated for 4–5 days in anaerobic condition at temp. 37°C in GENbox anaer (BioMerieux, Warsaw, PL). Anaerobes were cultured on fastidious anaerobe agar (FAA) (LAB M, Lancs, UK), anaerobic gram-positive bacteria on Columbia CNA Agar (BioMerieux, Warsaw, PL), anaerobic gram-negative bacteria on Schaedler'a Neo. Vanco agar (BioMerieux, Warsaw, PL), *Clostridium difficile* on clostridium difficile agar (BioMerieux, Warsaw, PL). Total count of clostridia was estimated after 1-h-incubation of equal portion of feces and ethyl alcohol, then diluted and inoculated on FAA. Lactobacilli were cultured in anaerobic condition at temperature 37°C for 72 h on Rogosa agar (DIFCO, Plymouth, UK). The detection limit of microorganisms was 3 log CFU g^{-1}.[10]

3.4. Statistical methods

Statistical analyses regarding overall bacterial colonization rate were performed for every feces' collection point (newborns, 3, 6, 12, 18 months) separately. To observe the changes in the colonization rate in relation to time we used Kruskal–Wallis test. Children given antibiotic treatment during the follow-up period were divided according to the route of antibiotic application: oral use (OU), intra-muscular injection (IMI) or both. These children and children who did not receive any antibiotic treatment were compared using Kruskal–Wallis test. Finally, we tested the general hypothesis of antibiotic influence on infantile intestinal flora based on the answer 'yes' for antibiotics used or 'no' for no antibiotic treatment during the observation period using Mann–Whitney *U* test. Breastfeeding as factor driving the development of intestinal flora was analyzed using Kruskal–Wallis test.

When recruited, children were classified into one of two subgroups depending on maternal atopy history. We categorized children into maternal atopy positive group when mother was suffering from atopic eczema, allergic rhinitis

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