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Full Length Research Paper

Initial microbial colonization in the alimentary tract of a new – born baby in different modes of parturition

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The intestinal microflora is a positive health asset that crucially influences the normal structural and functional development of the mucosal immune system. In the new born, the colonization of microbes in the alimentary system starts as soon as the womb comes out. A study on the early colonized bacterial species in a new - born will help to understand the immuno protective functions in the new-born. The initial colonization of bacterial population in the alimentary tract of a new-born baby varied according to the mode of child birth. In the alimentary tract of the normally delivered baby, beneficial probiotic microbial invasion in the intestinal region is high and such microbes are mostly from the mother. In a surgically (Caesarian) delivered baby, the alimentary tract receives mostly non beneficial microbes from the environment and hence such babies are susceptible to infection.

Key words: Lactobacillus, new-born, probiotic, immune system, nosocomial infection, Caesarian, normal child birth.

INTRODUCTION

The alimentary tract is an extremely complex living system that participates in the protection of the host through a strong defense against aggressions from the external environment. This defensive task is based on 3 constituents that are in permanent contact and dialog with each other: the microflora, mucosal barrier and local immune system (Baurlioux et al., 2003). The gut flora plays a major role against exogenous bacteria through colonization resistance. Bacteria in the gut helps to digest unutilized energy substrates, stimulating cell growth, repressing the growth of harmful microorganisms; training the immune system to respond only to pathogens and defending against some diseases (Wiest et al., 2004). It is logical that the intestinal microbial milieu influences immune reactivity to environmental antigens. Intestinal bacteria may impact on tolerance induction in several ways: First, bacteria may influence how intestinal epithelial cells function and thereby how foreign antigens are handled; Secondly, bacteria and/or bacterial products from the commensal microflora can be taken up across the mucosal barrier and influence antigens presenting cells in the Peyer's patches and intestinal Lamina propria;

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Thirdly, intestinal bacteria probably represent a significant proposition of all antigens which the immune system encounters (Bjorksten, 2004). As the intestinal microflora plays many vital roles in the human body, an attempt to evaluate the first colonising microbes in the sterile intestinal environment of new born infants will be much informative. The microbial consortia vary because of many factors (Baurlioux et al., 2003). Mode of birth, maternal flora, infant environment, probiotics, antibiotic drugs, age, diet composition etc., influences the colonisation of initial microbial population. Infants are born with a practically sterile gut, which is quickly colonised. The predominated source of initial colonization has a relationship with the mode of parturition. Hence in the present study an attempt has been made to evaluate whether the initial colonising microbes in the alimentary tract of just born infants have any relationship with vaginal and surgical (Caesarian) delivery.

MATERIALS AND METHODS

For the assessment of gut microflora in infants, mothers admitted in the obstetric department of a private maternity clinic where contacted and with the help of nurses and doctors, fecal samples and swabs where collected from new born babies. Fecal samples were collected in the initial five weeks after delivery. Freshly voided

Table 1. Media and culture conditions used for different bacterial groups.

Bacterial group	Culture medium (agar)	Conditions	Time (days)
Total aerobes and facultatives	Colombia blood aerobic	Aerobic	1
Staphylococci (coagulase neg. + *S. aureus*)	Staphylococcus	Aerobic	2
Enterococci (*E. faecalis* and *E. faecium*)	Enterococcosel	Aerobic	2
Total anaerobes	Brucella blood	anaerobic	3
Bacteroides	Bile esculin	anaerobic	3
Bifidobacteria	Beerens	Anaerobic	3
Clostridia	Brucella blood	Anaerobic	3
	Alcohol treated sample		
Clostridium difficile	CCFA	Anaerobic	3
Lactobacilli	Rogosa	Anaerobic	3

feces were collected by the parents and a sample was placed in a sterile, vented Petri dish using a sterile wooden spoon, which was then placed in a gas-proof plastic bag in which an anaerobic atmosphere was generated. Anaerobiosis was confirmed by inclusion of an anaerobic indicator in the bag. The samples were kept refrigerated until being transported to the laboratory where they were processed within 24 h after collection.

Bacterial culture

For fecal samples, an initial 10^{-1} was prepared by homogenizing 150 mg of faces obtained with a calibrated measuring spoon in 1.5 ml of sterile buffered peptone water. This dilution was further diluted lograthematically in sterile buffered peptone water from 10^{-2} to 10^{-9}. Appropriate dilutions were spread on selective and non-selective media for the isolation of anaerobic and aerobic or facultatively anaerobic bacteria (Table 1). Anaerobic cultures were performed for three days in anaerobic culture jars. The agar plates were pre-reduced for at least 24 h prior to use for the isolation of spore-formers (clostridia) a portion of the 1:10 sample dilution was mixed with equal proportions of 90% ethanol and incubated on a shaker at room temperature for 30 min. After this treatment, which kills vegetative cells, the sample was further diluted, placed on Brucella blood agar plates and incubated as previously described.

From appropriate dilution on selective and non selective agar plates, single colonies of different morphology were separately enumerated, gram-stained and sub-cultured for further identify-cation. All isolates were frozen at -70°C in Hogner's freezing medium. Bacterial isolates were identified by using standard biochemical test using Bergy's manual and API systems.

Bacterial identification

Facultative bacteria

Enterobacteria were defined as gram-negative rods growing aerobically on Drigalski agar plates. Isolates resembling enterobacteria were identified to the species level using the API20E biotyping System according to the manufacturer's instructions (API Systems SA, La Balme les Grottes, Montalieu-Vercieu, France).

Staphylococci were identified by growth on staphylococcus agar, typical gram stain appearance and a positive catalase reaction. Coagulase-positive staphylococci were identified as *Staphylococcus aureus*, while other staphylococci were defined as coagulase-negative staphylococci (CoNS).

Enterococci were identified by growth on enterococcosel agar, esculin hydrolysis and typical gram stain appearance. Yeasts were identified by growth on Sabouraud agar supplemented with penicillin and streptomycin, and typical gram stain appearance.

Anaerobic bacteria

Isolates from plates incubated anaerobically were checked for inability to grow under aerobic conditions (Colombia blood agar plates, 37°C), and isolates unable to produce visible growth in 24 h were defined as anaerobic bacteria. Weak growth under aerobic conditions was accepted for gram-positive rods, as certain species of *Lactobacillus* and *Bifidobacterium* are able to grow aerobically.

Anaerobic gram-negative rods growing on Bacteroides Bile Esculine agar were suspected to represent *Bacteroides* species. They were identified to the species level using the Rapid ID32A biotyping system (API systems), according to the manufacturer's instructions.

RESULTS AND DISCUSSION

The alimentary tract is a complex environment supporting a rich microflora. Gut flora are an important living biomass that generates intense metabolic activity mainly in the colon (Luckey et al., 1972). The various locations of the gastro-intestinal tract starting from the mouth to the anus have a variety of physiologic conditions and bacterial colonisation in each location varies. There is a very low population of bacteria in the stomach because it is highly acidic. The colon region harbors a higher bacterial colonization (10^{11} to 10^{12} colony forming units [cfu/g]) (Baurlioux et al., 2003). The population consists of predominate bacteria (>10^{9} cfu/g), sub dominate bacteria (between 10^{6} and 10^{9} cfu/g) also called repressed bacteria, which are the endogenous or resistant gut flora and bacteria in transit (<10^{6} cfu/g); depending on the number of bacteria ingested, the transits bacteria will be present in greater to lesser numbers (Baurlioux et al., 2003).

Table 2. Bacterial population in the fecal samples collected from new born infants born vaginally and by caesarian section (N= 25 from each mode of delivery).

Bacterial stain	Mode of delivery	Bacterial counts		
		1st week	3rd week	5th week
E. coli	Normal	1.61±0.2	2.94 ± 0.7	5.1 ± 0.6
	Caesarian	-	1.80 ± 0.2	3.4 ± 0.4
Klebsiella sp.	Normal	-	-	1.3 ± 0.2
	Caesarian	1.2±0.01	2.31 ± 0.2	4.3 ± 0.4
Other Enterobacteriaceae	Normal	-	-	1.3 ± 0.2
	Caesarian	1.31±0.08	2.14 ± 0.2	3.79 ± 0.4
Staphylococcus spp.	Normal	-	-	-
	Caesarian	-	-	2.31 ± 0.2
Bacterioids sp.	Normal	2.5±0.4	4.2 ± 0.4	5.8 ± 0.6
	Caesarian	-	1.2 ± 0.1	2.2 ± 0.4
Bifidobacterium sp.	Normal	3.4±0.7	6.7 ± 0.8	8.1 ± 0.7
	Caesarian	-	1.3 ± 0.4	2.4 ± 0.2
Lactobacillus sp.	Normal	3.7±0.3	6.6 ± 0.4	9.6 ± 0.7
	Caesarian	-	0.4 ± 0.2	2.1 ± 0.2

CFU counts (Log_{10}CFU) ± SD.

Some of these bacteria are useful to the host, such as bacteria of the genera Bifidobacterium and Lactobacillus. Other bacteria, to the contrary, are potentially pathogenic.

Colonisation of bacteria in the intestinal tract begins in the infant stage. Infants are born with a practically sterile gut, which is quickly colonized by bacteria from maternal flora in the case of vaginal delivery and environmental flora in the case of surgically delivered babies. The infantile flora evolves towards a normal adult flora over the first 24 months of life, but the course depends on infant's diets (Simon and Gorbach, 1984). Initially, in neonates' alimentary tract, the first bacteria to colonize depend on the innate repertoire (Huskin et al., 1985). However a number of other factors also influence colonization. It is now a firmly established fact that the flora of breast fed neonates, unlike the flora of infants who are being fed formula milk, are quickly dominated by Bifidobacetria (Harmson et al., 2000). However in the present investigation, mode of birth was also found to influence the initial colonization of microflora in the intestinal tract. Faecal analysis of neonates showed varied bacterial composition (Table 2). The flora of infants is very different from those of adults. Qualitatively, few species are present in the flora of infants; therefore infants are considered to be "immunocompromised" and are more likely to develop gastrointestinal infections and food allergies. The quality of the resident intestinal flora has a crucial effect on the intestinal immune system (Groulund et al., 2000). The presence of *Bifidobacterium* sp. in the fecal flora of breast feed children is associated with strong stimulation of the antirota virus IgA response compared with that observed in formula fed children (Salminen et al., 1998).

In the present study, the influence of mode of delivery (Caesarian section vs. vaginal delivery) and mother feeding were found to influence the early colonization of microbes in the gastrointestinal tract of neonates. Colonisation of different bacterial genera in the sterile gut of neonates varies. Infants born vaginally and breast feed had rapid colonization of *Escherichia coli*, *Bacteriods*, *Bifidobacterium* sp. and *Lactobacillus* sp. Wold et al. (2003) had also reported rapid colonisation of enterobacteriaceae group in guts of infants born through vaginal mode of delivery.

Colonization of *E. coli*, *Bacteriodes*, *Bifidobacteria sp*. and *Lactobacillus sp.* also varied in the neonate's alimentary tract according to the mode of delivery. Colonisation of these three genera of bacteria was more frequent in infants delivered vaginally than in those delivered by caesarian section.

E. coli and *bacteroides* are typical fecal bacteria that colonize the gut of infants born vaginally. In vaginally delivered infants, approximately 40% acquired *E. coli* and b*acteriods,* were transferred from the maternal perineal or fecal flora at the point of delivery (Wold et al., 2003). *E. coli* and *bacterioids* that find it difficult to thrive outside the intestinal tract were reported to colonise the gut of infants born vaginally from the early stage itself. In infants born by caesarian section, these bacteria were acquired or occurred later. It is believed that the microflora place a crucial role in immune maturation and development of oral tolerance. A number of scientific studies have shown that sterile animals are defective in oral tolerance that is, their immune system react strongly to foods and often fail

to response to infectious agents (Zetterstrom et al., 1994; Wilson et al., 1998). Hamilton (1999) showed that mice delivered by caesarian section and raised in germ-free incubators were far more susceptible to infection. Hanson (1997) suggested that active stimulation and direction of the infant's immune system takes place through the development of microflora. In the present study, vaginal-born infants had a good consortium of beneficial microbes that had reduced the infection of pathogens in this type of infants when compared with the section-delivered infants. Early colonization of microbes stimulates antibody response earlier and prepares the infants to protect them through immune response.

The present study concluded that vaginally delivered and breast fed infants were more resistant to bacterial infection when compared with infants delivered by caesarian section. Several workers had reported a relationship between intestinal colonization patterns in infancy with later allergic development. Colonisation of lactobacilli in the intestinal track of infants was found to be associated with decreased risk of having elevated total IgE levels by 18 months of age (Wold et al., 2003). It is believed that the exposure of intestinal epithelial cells or macrophages or dentric cells in the intestinal mucosa to lactobacilli promotes maturation of the regulatory process, perhaps regulatory T cells.

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REFERENCES

Bjorksten B (2004). Effects of intestinal microflora and the environment on the development of ashma and allergy Semin Immun 25: 257-270.

Baurlioux P, Berthold K, Franciso G (2003). The intestine and its microflora are partners for the protection of the host. Amj. Clin. Nutr. 78: 675-683.

Grönlund MM, Arvilommi H, Kero P, Letrhonen OP, Isolauri E (2000). Importance of colonisation in the maturation of humoral immunity in early infancy: A prospective follow-up study of healthy infants aged 0–6 months. Arch. Dis. Child Fetal Neonatal Ed. 83: 186–192.

Harmsen HJ, Wildeboer-Veloo AC, Raangs GC (2000). Analysis of intestinal flora development in breastfed and formula-fed infants by using molecular identification and detection methods. J. Pediatr. Gastroenterol. Nutr. 30: 61–67.

Hoskins LC, Augustines M, McKee WB, Boulding ET, Kriaris M, Niedermeyer Mucin G (1985). Degradation in human colon ecosystems. J. Clin. Invest. 75: 944–953.

Luckey TD (1972). Introduction to intestinal microecology. Am. J. Clin. Nutr. 25: 1292–1294.

Salminen S, Bouley C, Boutron-Ruault MC (1998). Functional food science and gastrointestinal physiology and function. Br. J. Nutr. 80: 147–171.

Simon GL, Gorbach SL (1984). Intestinal flora in health and disease. Gastroenterol. 86: 174–193.

Wiest R, Garcia Isac G (2004). Gut microflora in the pathogenesis of complications of cirrhosis best practice and research clinical gastro enterology 18(2): 353-372.

Wilson AC, Forsyth SC, Greene SA, Irvine L, Hagau C, Howie PW (1998). Relation of infant diet to childhood health; seven year follow up of children in Dundee infant feeding study. British Med. J. 316: 21-25.

Wold A (2003). Impact of intestinal microflora on allergy development (Allergy flora) Project report QLK-CT-2000-00538. Dept of Bacteriol., Guldhedsgatan Goteborg. Swedan 46: 10-413.

Zetterstrom R, Bennett Nord KE (1994). Early infant feeding and micro-ecology of the gut. Acta Paediatrica Japonica 36: 562-571.