

Knowledge of the Digestive Microflora: Evaluation of Breastfeeding on the Establishment of the Gut Microbiota of the Newborn

Souad Bouchachia^{1,2}, Hassiba Mahdjoub Bessam^{1,2}

¹Department of Biology, Faculty of Natural Sciences & Life, Djillali Liabes University of Sidi Bel-Abbes, Sidi Bel-Abbes, Algeria

²Laboratory Eco Development of Areas, Faculty of Natural Sciences & Life, Djillali Liabes University of Sidi Bel-Abbes, Sidi Bel-Abbes, Algeria

Email address:

tek6-6hl@hotmail.fr (S. Bouchachia)

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Abstract: At birth, the newborn has no bacteria in its gut. This last is rapidly colonized by microbial flora (microbiota) from, mainly of the mother and environment. The main objective is the study of the influence of breastfeeding in the establishment of the intestinal microbial flora in the newborn. The secondary objective of this work was to analyze the bacterial diversity in feces of breast-fed infants and to compare it with that of formula-fed ones. Five pairs of mother-child and 5 fed formula have participated in the study. The Samples were taken at 1, 7, 30 and 90 days and plated out on various culture media. The present study shows that breast milk plays a major role in the development of the intestinal microbiota of the child. The results of this comparative study showed that the fecal matter of a mother breast-fed infants are more rich in probiotics and less rich in pathogenic bacteria than infants receiving infant formula.

Keywords: Microbiota Intestinal, Newborn, Breastfeeding, Breast Milk, Infant Formula, Probiotic, Lactic Acid Bacteria

1. Introduction

The human body is inhabited by numerous bacteria located mainly in the intestine known as the gut microbiota [1]. The intestinal microbiota contains approximately 10^{14} bacteria in 4 bacterial phyla: the Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria [2]. Intestinal bacteria colonize our gut in the first hours of life and form an early ecosystem remarkably stable throughout life [3]. The newborn, sterile at birth, is colonized with flora from contact with his mother and environment [4]. Human milk represents one of the main factors that play a critical role in influencing infant's microbiota composition, also being a direct source of microbes [5]. Research over the past decade has confirmed the essential role of breast milk in the establishment a healthy intestinal microbiota within the infant [6].

The main objective of this work is the study of the influence of breastfeeding on the development of the intestinal microbial flora of newborn. The secondary

objective of this study is to identify the constituent strains of bacteria of the intestinal flora in infants breastfed and bottle, in order to control the behavior of different microbial flora.

2. Materials & Methods

2.1. Plan of Study and Subjects

This study was conducted between May 2014 and December 2015. Five mother-child pairs were recruited to establish Mother-Child. Five healthy women (mean age 33.2 ± 4.53) were enrolled after delivery in the establishing Mother-Child.

2.2. Collection of Sample

Breast milk samples (5 women) and feces of infants (5 infants) were collected at 1.7, 30 and 90 days. Breast-milk samples were obtained by manual expression after cleaning the nipples and areola by wiping with a swab soaked in

sterile water and discarding the first drops.

All Infants: 5 Newborn breastfed maternally (3 Boys / Girls 2) and 5 infants fed formula were born in the hospital neonatal unit after an uncomplicated pregnancy. Infants were vaginally delivered, at a gestational age of 39.2 weeks. None of the mothers or babies received antibiotic therapy during the sampling period.

2.3. Microbial Counts

2.3.1. Microbiological Analyzes

Samples were taken to the laboratory at 4-5°C using a cooler and stored in the laboratory refrigerator at 4°C until use.

Lactobacilli were enumerated on MRS medium (Man-Rogosa-Sharpe) incubated at 37°C for 72 h anaerobically. The enumeration of streptococci and enterococci were performed on M 17 medium incubated at 37°C and 42°C for 48 hours under aerobic conditions [7].

Bifidobacteria were enumerated on MRS medium supplemented with L-cysteine (0.5 g / liter), which were incubated anaerobically at 37°C for 48 h [8].

Staphylococci are counted on Chapman agar. The incubation was carried out 48 hours at 37°C. The enumeration of yeasts and molds on the classic Sabouraud medium. Incubation was carried out for 5 days at 37°C [9].

2.3.2. Identification of Isolates

Regarding the different identifications and confirmations of lactic acid bacteria, using two first types of galleries: API 20 STREP (API 20 Strep, Bio Merieux, France) and API 50 CHL gallery (API 50 CHL, Bio Merieux, France) with their boxes of reagents. API Staph system was evaluated as a means for identifying staphylococci species and for identification of enterobacteria, using the API 20 E. Then we used the identification catalogs for confirmation and then the API Web™ software (Bio Mérieux, France).

2.3.3. Statistical Analyses

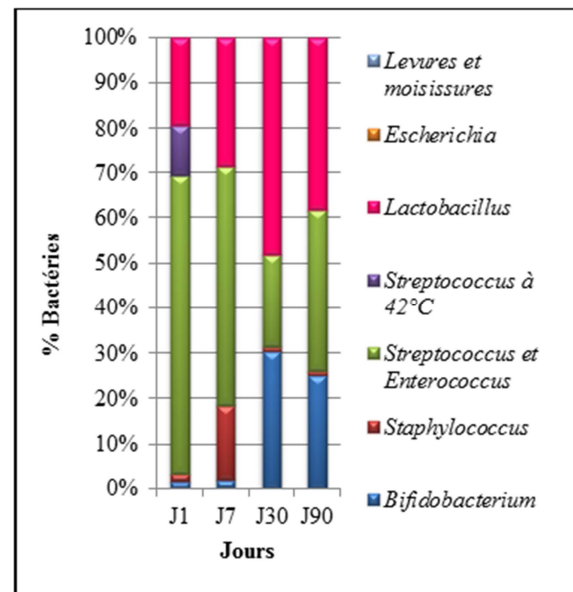
All bacterial counts were analyzed after log10 transformation. Bacteria levels in samples of breast milk and infant faeces who received breast milk and those who received formula milk was compared using the *t*-test. Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and the Excel 2007 software. The statements of statistics were considered to be significant when $P < 0.05$.

3. Results

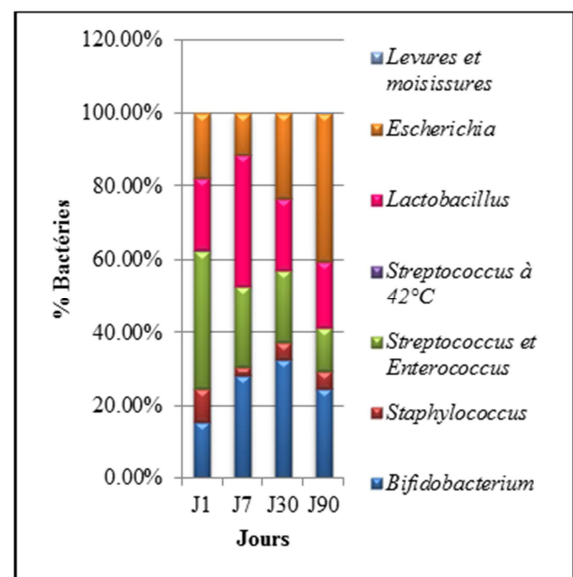
3.1. The Influence of Breastfeeding in the Establishment of the Microbial Flora of the New Born

From day 1 of bacterial counts, there is a microbial diversification in milk after microbiological analyzes revealed the presence of *Streptococcus* and *Enterococcus* at 37°C to value 3 log CFU / g to 3.57 log CFU / g with a percentage of 65.96% and 11.31% at 42°C, respectively. The presence of *Streptococcus* in fecal samples first day varies

between 2.69 log CFU / g to 3.84 log CFU / g, a non-significant result was observed ($P = 0.83$).



(a)



(b)

Figure 1. The percentages of isolation of different bacterial genera in the breast milk (A) and infant feces (B) at different sampling points analyzed.

Bifidobacterium rate in milk varies between 1.55 log CFU / g up to 2 log CFU / g with a percentage of 1.56% and faeces varies between 0 to 3.61 log CFU / g with a percentage of 15.36%. *Lactobacillus* rate ranges from 0 to 3.40 log CFU / g with a percentage of 19.39% in breast milk and 0 to 3.74 log CFU / g in infant faeces, with a percentage of 19.50%. It does there was no significant difference ($P > 0.05$) on bifidobacteria and *lactobacillus*.

Concerning pathogenic bacteria *Escherichia* absence is observed in milk and percentage of 18.00% in the faeces, a statistically significant ($P = 0.009$). The presence of

Staphylococcus to a value of 1.86 to 2.17 log CFU / g with a percentage of 1.76% in the milk and 0 to 3.47 log CFU / g with percentage of 9.12% in the feces.

On the seventh day there is an increase in the *Bifidobacterium* and *Lactobacillus* rate of 28.17% and 35.72% respectively in infant faeces, the difference is not statistically significant ($P = 0.98$; $P = 0.55$)

The 30th day reduction in *Streptococcus* rate observed in milk and feces with a percentage of 20.50%, 19.90% respectively. An increase of *Escherichia* in faeces (23.25%). It is there fore considered statistically significant ($P = 0.009$)

The 90th day there is a lack of yeasts and molds in milk

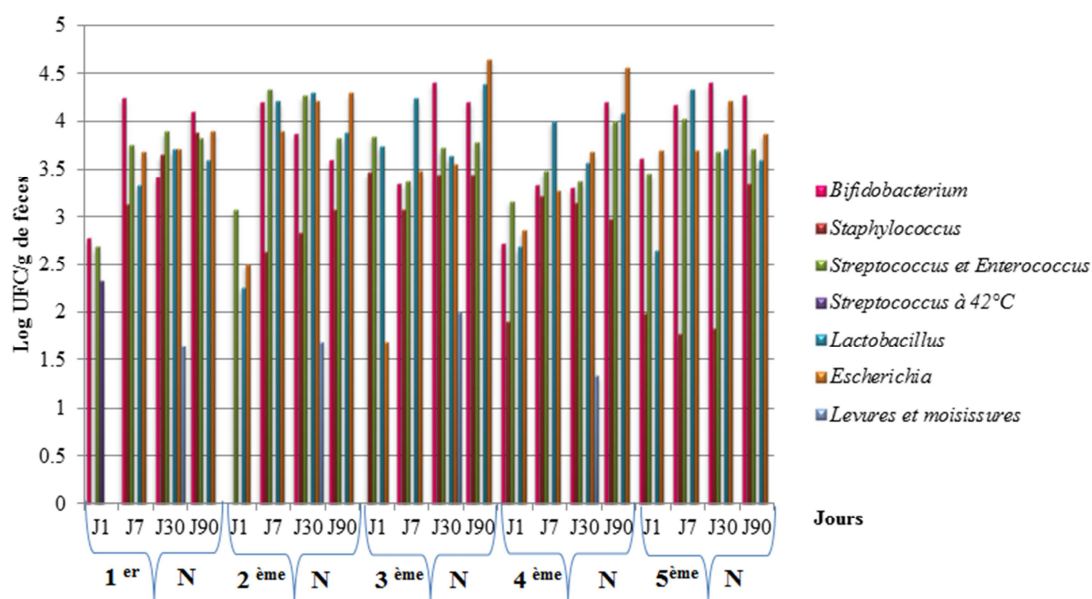
and presence in feces ($P < 0.05$), about pathogenic bacteria a significant result was observed ($P < 0.05$) (Figure 1).

The strains isolated from breast milk are:

Leuconostoc spp, *Streptococcus anginosus*, *Streptococcus salivarius*, *Lactococcus lactis ssp cremoris*, *Staphylococcus hominis*, *Bifidobacterium spp*, *Lactobacillus paracasei ssp paracasei* 1, *Lactobacillus acidophilus* 1, *Lactobacillus delbrueckii ssp delbrueckii*, *Leuconostoc mesenteroides ssp cremoris*.

Among the strains isolated from feces for infants, there is a predominance of *Enterococcus faecalis* and *Enterococcus faecium*.

3.2. The Evaluation of the Intestinal Flora of Breastfed Infants of a Mother and Breastfed Infants with Formula Milk



N: Infants

Figure 2. Counting result of fecal flora of the 5 infants breastfed maternally.

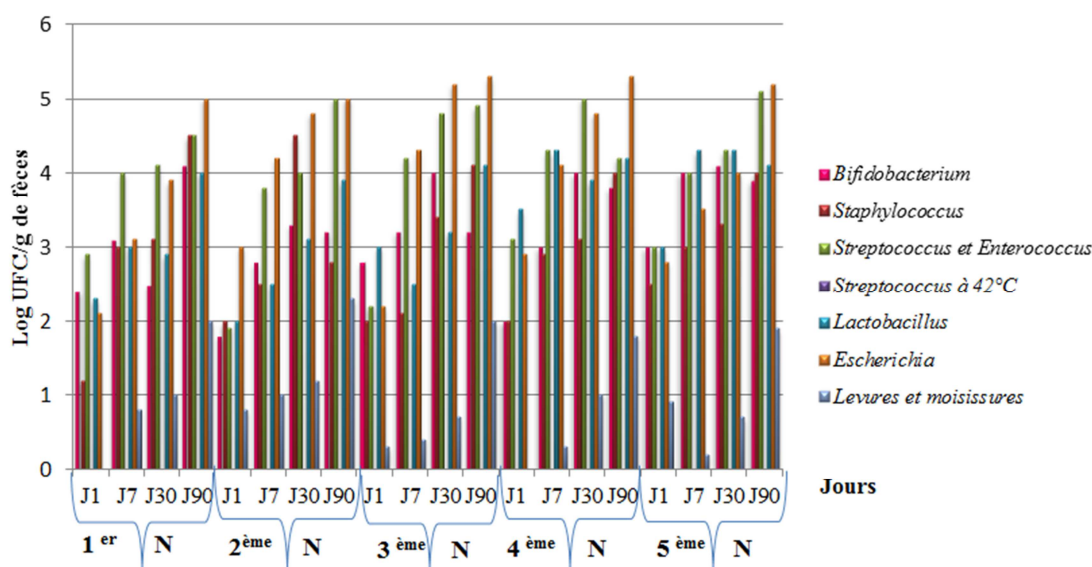


Figure 3. Counting result of fecal flora of breastfed infants 5 artificially.

Maternally breastfed infants, bifidobacteria, having their maximum during their first month of life with values 4.4 log CFU / g of feces (Figure 2). In infants receiving infant formula, bifidobacteria are at rates varying between 2 to 4 log CFU / g for 90 days.

However, the bacterial genus *Escherichia* is in faeces artificially breastfed infant during the first month at high rates around 5.2 log CFU / g. Interestingly, in the sampling point first found statistically significant differences in the levels of *Escherichia* between maternally breastfed and those who did not after the 30th day and 90th day (3.86 ± 0.3 , 4.54 ± 0.5 ; 4.25 ± 0.36 , 5.16 ± 0.15 , respectively)

Low quantities of the *Staphylococcus* genus are recorded on the first day of life for all breastfed infants maternally at 0 Log CFU / g of feces first infant then this amount increases over the days to neighboring values 3.88 log CFU / g after the third month of life. However staphylococci are at much higher for infants fed formula milk and it increases over the month to reach higher values of about 4.5 log CFU / g on the 90th day of the first infant receiving artificial milk. (Figure 3).

4. Discussion

Improve our understanding of gut microbiota could be useful to better understand the roles of microbial strains in promoting the health of infants [10]. Colostrum and milk play a key role in the initiation, development and composition of infant intestinal microflora [11].

The interesting observation of West et al. [12] that breast milk is not sterile even when collected aseptically. An independent and dependent analysis of the culture of human milk samples revealed the presence of the genus *Streptococcus* and *Staphylococcus*, which corresponds to the first colonizers of the digestive tract [13].

Martin and his colleague (2003) showed that the same strains of lactic acid bacteria in breast milk are also present in the faeces of the infant [14].

This study confirms our results find that the same lactic strains such as *Bifidobacterium spp*, *Lactobacillus paracasei ssp paracasei 1*, *Lactobacillus acidophilus 1*, *Lactobacillus delbrueckii ssp delbrueckii*, *Leuconostoc mesenteroides ssp cremoris* found in breast milk and infant feces.

L. delbrueckii and *L. reuteri*, were among the most frequently isolated bacteria in fecal samples obtained from Swedish infants during the first year of life [15].

In the works of De Leoz et al. [16] the rate of *Bifidobacterium* is noticed after the 13th week of life infants, confirming the absence of *Bifidobacterium* in some infants in this study.

The microbiological analysis of breast milk levels of *Streptococcus* reveals the presence of high levels, agree with Solis et al. [17] with a percentage of 65% *Streptococcus* is the first day and 50% on the 90th day.

Indeed, Schultz et al. [18] confirmed such a transmission of probiotic strains from mother to child during birth; although the specific probiotic strain was present in very low

numbers (10^4 – 10^5 CFU/g feces) for most infants, it persisted at least until the age of 6 months.

A recent hypothesis suggests a novel way of mother–neonate communication, in which maternal gut bacteria reach breast milk via intestinal translocation and blood carriage, describing an internal entero-mammary pathway to influence neonatal gut colonization and maturation of the immune system. This novel pathway of bacterial transfer would support the addition of carefully selected bacteria from mother's breast milk to formula and opens new windows of opportunity for designing probiotics tailored to the infant. [19]

A few hours after birth, babies are colonized by bacteria and develop their normal bacterial microflora [20, 21], by type of food and closely associated with the environmental microflora [20].

Enterococcus faecalis was the second bacterial species prevalent in fecal samples provided by nursing infants, but it was present in all samples of those bottle-fed [22]. This study confirms our findings that finds an abundance of *Enterococcus faecalis* in fecal samples in both groups.

The present study shows the abundance of potentially pathogenic bacteria such as *Escherichia*, *Staphylococcus* in feces of infants receiving infant formula compared to infants breastfed maternally feces is due not only to the rich breast milk by specific factors that promote the growth of potentially beneficial bacteria and inhibit the potentially pathogenic germs.

Similarly, the work of two researchers Bezirtzoglou and Stavropoulou [23] show that breastfed infants maternally an acidic environment in the intestinal lumen "acetate buffer" suggesting that this acid has a bacteriostatic effect on potentially pathogenic germs especially against bacteria gram-negative.

5. Conclusion

The intestinal microbiota is now considered a full body finely interacting with the host. Research on these commensal bacteria in recent years is growing but many roles remain to be discovered [24]. Our results indicate that breast milk contains *Lactobacillus* and *Bifidobacteria* that could contribute to the establishment and development of microbial flora in the newborn. The present study shows that breast milk plays a major role in the development of the intestinal microbiota of the child. The results of this comparative study showed that the fecal matter of a mother breastfed infants are more rich and less rich probiotic bacterial pathogens that infants receiving infant formula. Micro organisms isolated in this study may be promising strains for inclusion in infant formula. The application of molecular ecology tools intestinal microbiota led to very significant improvements in our understanding of this ecosystem, in terms of composition and dynamics of species diversity.

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