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Impact of Delivery Mode on the Intestinal Flora of Newborns in the Sidi-Bel -Abbès Region in Algeria

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ABSTRACT

In the present study, we were interested in the impact of delivery mode on the establishment of the newborns' gut microbiota. We characterized the isolated bacteria from the infants' stools from the population of western Algeria (Sidi- Bel- Abbès). Details regarding the mode of delivery have been collected.

Different approaches were used for the identification of the bacterial strains isolated. A first screening of the isolates was carried out on the basis of phenotypic criteria.

The results of the statistical analysis showed a homogeneous distribution, for the four genera, between the stool samples of infants born by cesarean section and those born vaginally; *Enterococcus* and *Staphylococcus* are the dominant genera in the infants' stool born by cesarean section (73.3% and 40% respectively) and in the infants' stool's born vaginally (73.3% and 13.3% respectively), *Lactobacillus* (60% and 86.67% respectively) and in small proportion the genus *Streptococcus* (13.3% and 40% respectively). Our results showed that the mode of delivery plays an important role in the establishment of the intestinal microbiota in infants. Other external and internal factors related to the host can also influence this process.

According to our literature investigations, this preliminary study is the first to compare the bacterial communities of the stools of infants in the two groups of the Algerian population. To complete our study, it would be interesting to expand the sampling and identify the different bacterial strains in order to more precisely determine the bacterial diversity in our population.

INTRODUCTION

Our digestive tract is populated by hundreds of species of bacteria, forming a complex ecological community, referred to as the gut flora or microbiota. It is the most important flora of the organism and it plays a key role in the metabolic functions essential to digestion and forms a barrier against the implantation of pathogenic germs.

The balance of this intestinal ecosystem is sensitive, there are many causes that can hinder proper digestion and lead to dysbiosis and intestinal hyperpermeability. An imbalance in the intestine and its flora has significant functional consequences and is involved in many digestive pathologies (Sokol, 2014).

The implantation of the intestinal microbiota at birth and its maturation in the first years of life is an essential step that leads to the development of an intestinal flora specific to the host. (Dominguez-Bello *et al.*, 2010)

There are many internal and external host factors that influence the implantation of the gut microbiota. Thus, maternal microbiota, in particular vaginal and fecal microbiota, are the primary sources of bacteria. Food is a daily repeated intake of microorganisms too, especially breast milk (Scholtens *et al.*, 2012).

In this context, we conducted this study to determine the impact of the delivery mode on the composition of the newborns' intestinal flora in the West Algerian population (Sidi -Bel- Abbe's city).

We have collected newborns' stool samples from a group of 30 infants. The culture media: MRS (Man-Rogosa-Sharpe), and CBA (Columbia Blood Agar), allowed us to select 4 bacterial genera whose identification could not be carried out due to the lack of certain reagents (oxidase test).

Finally, statistical analysis was carried out in order to compare the distribution of bacterial communities in the stool of babies born by cesarean section and those born vaginally and to determine the impact of the delivery mode on the development of the newborn intestinal microbiota.

MATERIALS AND METHODS

Place of Work and Purpose:

This work was carried out in the

laboratory of general microbiology at the Faculty of Natural Science and Life, Djilali liabés University of Sidi-Bel-Abbés, Algeria, during the period from April to June 2021.

The samples (newborn's stool) were collected at the maternity neonatal service in Sidi-Bel-Abbés, Algeria.

The aim of our study was to compare the distribution of the bacterium genera (*Enterococcus*, *Lactobacillus*, *Staphylococcus* and *Streptococcus*) in the newborn as well as to study their prevalence according to the delivery mode.

Sampling Plan:

Stool samples from 30 breastfed newborns 0-1 days old to perform isolations of their microbial flora. 15 newborns were born vaginally, while 15 were delivered by Caesarean section. All were full-term and were not given antibiotics treatments. All participants were in good health and without any prenatal problems.

Sampling was carried out under sterile conditions. The feces were collected with sterile swabs and were kept cool until delivery to the laboratory where they were immediately processed.

Isolation and Purification of Bacterial Strains:

The samples were cultured on MRS medium (Man-Rogosa-Sharpe, Oxoid) supplemented with 0.05% Cysteine-HCL and CBA medium (Columbia blood agar, Biomerieux). Agar Petri dishes were incubated at 37 ° C under anaerobic conditions for 48-72 hours, (Lagier *et al.*, 2015).

Counting of Lactic Acid Bacteria:

The counting of lactic acid bacteria was carried out on the MRS medium. The stock solution was prepared by taking 1 g (feces) from each sample which was added to 9 ml of sterile physiological water. Serial dilutions ranging from 10⁻¹ to 10⁻³ were made from these stock solutions from

the cesarean and vaginal deliveries newborn stools. 0.1 ml was taken from the dilution chosen 10^{-3} and spread in a Petri dish of MRS medium. Then, the dishes were incubated at 37°C under aerobic conditions for 48-72 hours. Colonies were counted for each dish to determine the number of CFU/g using the following formula:

Calculation of the bacterial concentration N in CFU per g of product (Reuben *et al.*, 2019):

$N = \text{number of colonies} \times 1 / V \times 1 / D$

CFU: Colony Forming Units

V: the inoculation volume

D: the dilution is taken into account.

Purification:

The isolated colonies are subcultured alternately on liquid and solid medium until purification. The strain purity was verified by microscopic observation.

Phenotypic Characterization of Bacterial Strains:

Morphological Study: The bacterial morphology study is the first act performed to identify bacteria. It allows a preliminary orientation of the diagnosis.

Macroscopic Characterization: The macroscopic examination was carried out to describe the appearance of the colonies (shape, size, outline pigmentation, viscosity, etc.)

Microscopic characterization: A smear for Gram stain was prepared from an isolated colony. Once carried out, it is observed under an optical microscope (Thairu *et al.*, 2014).

Biochemical Study:

Catalase Test: Catalase is an enzyme found in most strict aerobic and facultative aerobic bacteria. To carry out this test 2 to 3 drops of 10 volumes of hydrogen peroxide were placed on the slide and mixed with a few cells from the colony using a sterile pipette. A gas release means that the strain is catalase

+, the absence of gas means that the strain is catalase negative (Leveau and Bouix, 1993; Delarras, 2007).

Statistical Analysis:

Statistical analyzes were performed using IBM SPSS version 24 software. The chi-square test was used, to compare the distribution of bacterial genera in the newborn stools (NC, NB) on the other hand, this test was used to study the independence between different factors and the prevalence of the bacterial genders in these two samples. The test was considered significant if P value <0.05. All data was described in number (n) and percentage (%).

RESULTS AND DISCUSSION

Enumeration of Lactic Acid Bacteria:

The lactic bacteria enumeration was obtained on the MRS medium (Fig.1). The mean count values oscillate according to the nature of the sample, with a significant difference (P-value <0.001) of 6.38 to 7.32 log₁₀ CFU / g for the newborn feces samples.

Isolation and Identification of Bacterial Strains:

Phenotypic Characterization of Bacterial Strains: Colonies of different morphologies were isolated from the newborn stool samples and were examined under a light microscope for cell morphology as well as reaction to gram stain. The tests for catalase and oxidase activity were carried out subsequently.

From the results obtained we selected and purified 100 different strains. (50 from newborn stool samples delivered by caesareans and 50 from newborn stool samples delivered vaginally).

Morphological Study and Macroscopic Characterization:

After 48 to 72 hours of incubation, bacterial growth is naked-eye visible and represented as bacterial

colonies on the surface of the agar media. On the MRS medium, the isolated colonies measured approximately 1 mm, they are white and transparent, round in

shape with a regular or irregular outline. The appearance is also different between isolates, it is either smooth or rough (Fig. 2).

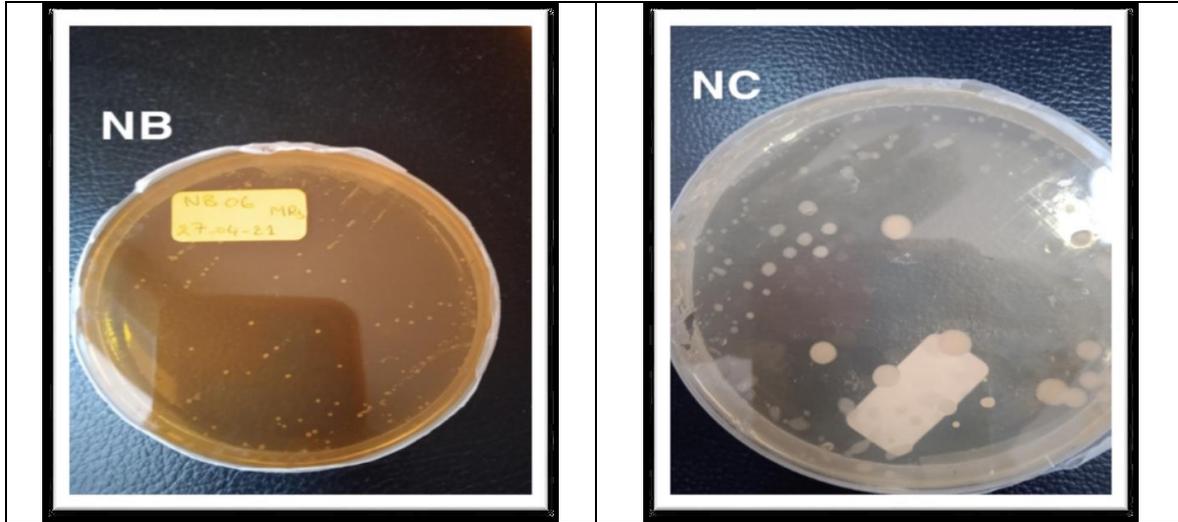


Fig. 1: Macroscopic appearance of the *Lactobacillus* in MRS medium

On Columbia blood agar (CBA), we obtained small round colonies with a regular outline, of different colors (white,

yellow, beige), opaque or shiny, surrounded or not by a clear halo (hemolysis) (Fig. 2).

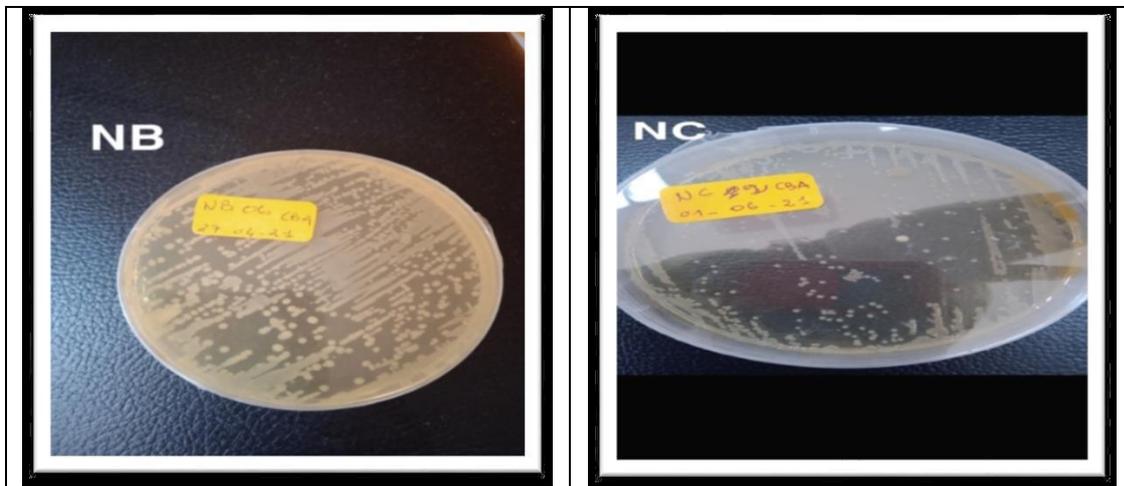


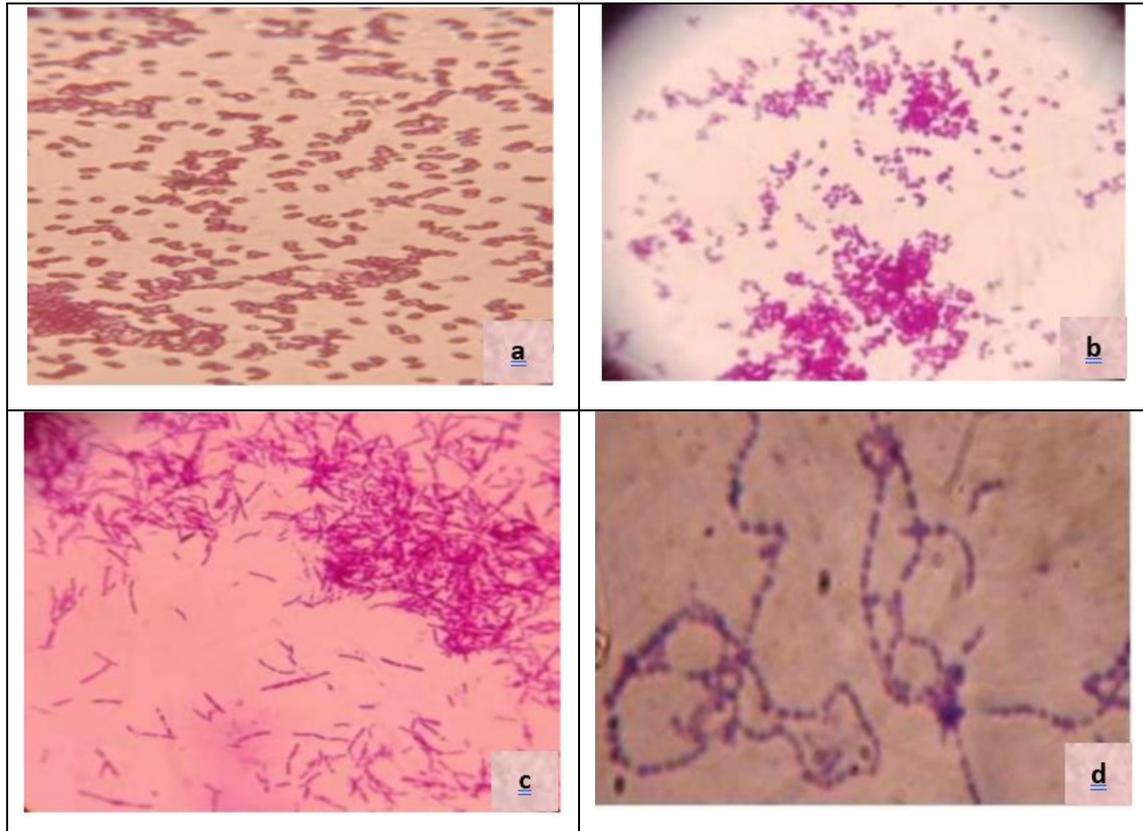
Fig.2: Macroscopic appearance of the *Lactobacillus* in CBA medium

Microscopic Characterization: After the Gram stain, microscopic observation shows that the strains studied are Gram-positive bacteria with two cell forms (cocci and bacilli). The association method depends on the strain tested (Fig. 3). Bacteria in the form of cocci were presented in 72% of NC and 66% of NB.

They are arranged either in pairs and short chains (*Enterococcus*), in long chains (*Streptococcus*), or in clusters (*Staphylococcus*). On the other hand, the bacilliform is represented by the genus *Lactobacillus* with 28% for NC and 34% for NB, and are arranged in pairs or chains (Table .1).

Table 1: Phenotypic characteristics of bacteria isolated from infants' stool samples (NC and NB).

	Morphology		Gram Stain		Catalase test	
	Cocci	Bacilli	+	-	+	-
NC (n=15)	36	14	50	0	9	41
% (NC)	72%	28%	100%	0%	18%	82%
NB (n=15)	33	17	50	0	2	48
% (NB)	66%	34%	100%	0%	4%	96%

**Fig. 3:** Microscopic observation of the bacteria after Gram staining ($\times 1000$) *Enterococcus* (a), *Staphylococcus* (b), *Lactobacillus* (c), *Streptococcus* (d).**Biochemical Study:**

Catalase Test: From the samples studied, 11 (09 NC and 02 NB) reacted in contact with oxygenated water by effervescence due to the release of O_2 (Fig. 4); they are

positive Catalase. The remaining 89 strains (41 of NC and 48 of NB) did not give any reaction; they are negative Catalase.

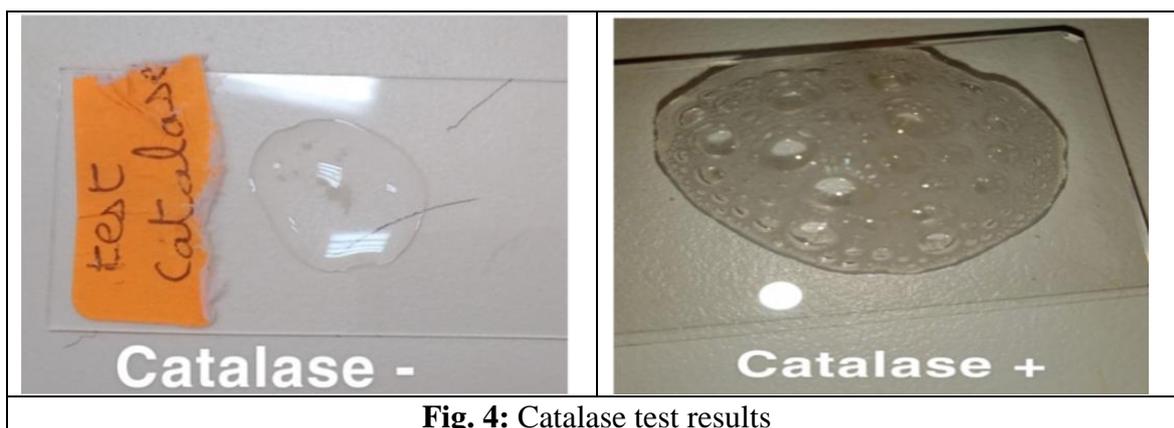


Fig. 4: Catalase test results

Statistical Analysis:

Bacterial Genera Distribution in The Newborn Groups:

The statistical approach allowed us to compare the genera distribution (*Enterococcus*, *Lactobacillus*, *Staphylococcus* and *Streptococcus*) in the stools of infants as well as to study their

prevalence according to the delivery mode.

By comparing the stool flora of infants born by cesarean section (NC) and those born vaginally (NB), no significant difference in the colonization rates of the 4 genera of the bacteria studied was observed ($P > 0.05$), (Table .2).

Table 2: Bacteria distribution in the two groups

Gender	Presence or absence	Samples				Khi ²	p
		NC		NB			
		n	Percentage	n	Percentage		
<i>Staphylococcus</i>	Presence	6	40	2	13,3	*2,727	0,099
	Absence	9	60	13	86,7		
<i>Enterococcus</i>	Presence	11	73,3	11	73,3	*0,000	1,000
	Absence	4	26,7	4	26,7		
<i>Lactobacillus</i>	Presence	9	60	13	86,67	*2,727	0,099
	Absence	6	40	2	46,7		
<i>Streptococcus</i>	Presence	2	13,3	6	40	*2,727	0,099
	Absence	13	86,7	9	60		

* Cramer's Phi V = 0.302

Enterococcus and *Staphylococcus* are the dominant genera in the cesarean section newborn stool (73.3% and 40% respectively) and in the vaginally newborn stool (73.3% and 13.3% respectively),

Lactobacillus is the second part of the microbiota (60% and 86.67% respectively) and in small proportion the *Streptococcus* genre (13.3% and 40% respectively) (Fig .5).

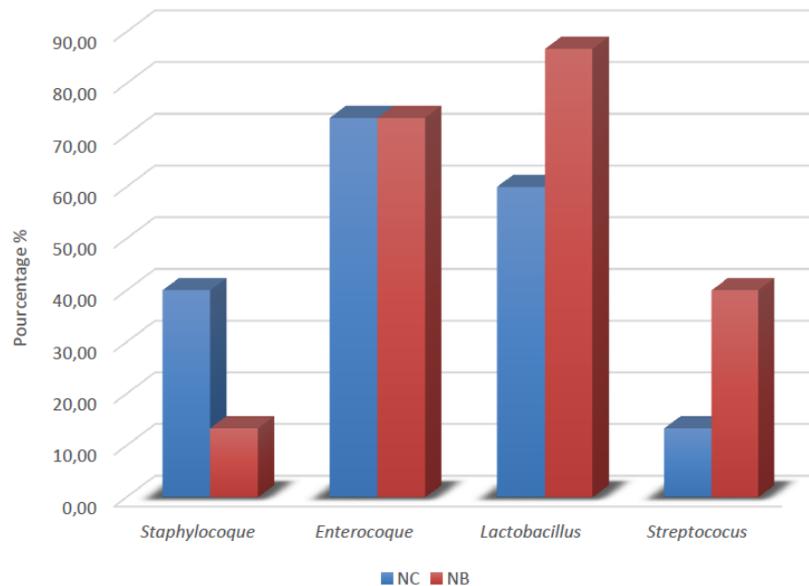


Fig. 5: Bacteria distribution in the two groups

DISCUSSION

The intestinal flora or microbiota is the set of microorganisms (mainly bacteria) that colonize the human digestive tract. Each individual has a unique gut microbiota, as many factors influence its development and composition. (Ajslev *et al.*, 2011). Early childhood is a key period. In fact, after the newborn's first contact with his environment, external microorganisms invade the digestive tract. The young child thus presents in his first years an increasingly various and complex intestinal microbiota which will be stabilized around the age of 2 to 3 years. This process of microbial "colonization" could have a profound influence on health status throughout life (Reyman *et al.*, 2019).

In this study, we were interested in the impact of delivery mode on the establishment of intestinal flora in newborns. We isolated and characterized the bacteria from the newborn stools from a population of western Algeria.

The counting of lactic bacteria in the newborn stools was carried out on

MRS medium. The results showed that the number of bacteria was variable and specific to each individual. The mean value of bacteria in the cesarean section newborn stools was 7.32 log₁₀ CFU/ml and 6.85 log₁₀ CFU/g in the vaginally newborn stool. These results are consistent with the findings of other similar studies (Scholtens *et al.*, 2012).

The statistical analysis showed that there was a homogeneous distribution of the genera studied in the infant's stools. The vaginally newborn fecal flora were compared to the cesarean section newborn ones. No significant difference was observed in the distribution of the genera *Enterococcus*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus* ($p > 0.05$). The prevalence of these genera was independent of the route of delivery ($p \leq 0.05$).

The impact of the delivery mode on the newborn intestinal flora has been extensively studied in recent years. Several studies have found greater bacterial diversity in infants born vaginally as well as delayed colonization in infants born by cesarean section (Azad *et al.*, 2016).

In this study, we recorded that the presence of *Staphylococcus* was associated with the delivery route with a very high colonization rate in children born by cesarean section (40%). This result is in agreement with those of Dominguez-Bello *et al.* (2010), who found that the dominant taxa present in newborns reflect the mode of delivery. They found that infants born by cesarean section exhibited bacterial communities similar to those found on the skin surface, dominated by *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp; while vaginally delivered infants harbored bacterial communities resembling their mothers' vaginal microbiota, dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp (Dominguez-Bello *et al.*, 2010).

Recently, the same authors, revealed that by exposing children born by Caesarean section to vaginal secretions, their microbiota is significantly similar to that of infants born vaginally compared to unexposed infants (Dominguez-Bello *et al.*, 2016). This simple gesture could constitute a new alternative to restore the microbiota of children born by cesarean section.

CONCLUSION

In the present study, we characterized some species present in the stools of breastfed infants. We found a similarity in some bacterial communities of infants born vaginally and those born by cesarean section.

To compare the intestinal flora according to the different factors studied, we suggest increasing the number of samples for better results.

To our knowledge, this is the first study to compare the flora of the stools of infants in the Algerian population. Thus, we propose to carry out further studies to explore other genera such as Bifidobacteria and to characterize the isolates at the strain level. This could confirm or invalidate our results on the role of the various factors studied in the

establishment of the intestinal flora of newborns.

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ARABIC SUMMARY

تأثير طريقة الولادة على البكتيرية المعوية لحديثي الولادة بولاية سيدي بلعباس بالجزائر

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في هذه الدراسة، اهتمنا بتأثير طريقة الولادة على تكوين ميكروبات الأمعاء عند الأطفال حديثي الولادة. للقيام بذلك، قمنا بتمييز البكتيريا المعزولة من براز الأطفال من سكان غرب الجزائر (سيدي بلعباس) كما تم جمع المعلومات المتعلقة بالولادة. تم استخدام طرق مختلفة لتحديد السلالات البكتيرية المعزولة كما تم إجراء الفحص الأول للعزلات على أساس المعايير الظاهرية.

أظهرت نتائج التحليلات الإحصائية توزيعًا متجانسًا للأجناس الأربعة بين عينات براز الأطفال المولودين بعملية قيصرية وأولئك المولودين عن طريق المهبل. المكورات المعوية والمكورات العنقودية هي الأجناس السائدة في براز الرضع المولودين بعملية قيصرية (73.3% و 40% على التوالي) وفي براز الرضع المولودين عن طريق المهبل (73.3% و 13.3% على التوالي)، Lactobacillus (60% و 86.67% على التوالي) وفي أما Streptococcus فقد تواجدت بنسب ضئيلة (13.3% و 40% على التوالي)، تُظهر نتائجنا أن طريقة الولادة تلعب دورًا مهمًا في تكوين الجراثيم المعوية عند الرضع الذين يرضعون رضاعة طبيعية كما أن للعوامل الخارجية والداخلية الأخرى المتعلقة بالعائل أيضًا تأثيرًا على هذه العملية.

وفقًا لأبحاثنا النظرية، فإن هذه الدراسة الأولية هي الأولى التي تقارن التجمعات البكتيرية لبراز الرضع في مجموعتي السكان الجزائريين. لاستكمال دراستنا، سيكون من المهم توسيع نطاق أخذ العينات وتحديد السلالات البكتيرية المختلفة من أجل تحديد التنوع البكتيري في مجتمعنا بشكل أكثر دقة.

الكلمات المفتاحية: طريقة الولادة؛ الجراثيم المعوية؛ بكتيريا؛ براز الرضع