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# Phenotypic and Genotypic Identification of Bacteria from Women Breast-Milk and the Feces of their Childs in the Western Region of Algeria

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**Breast-milk is an important source of bacteria for the colonization of the infant's gut. The aim of our study was to isolate and identify bacteria from samples of breast-milk of 32 women and from fecal samples of their breast-fed infants. Antimicrobial activity of isolates was also performed. A total of 155 isolates were characterized by phenotypic tests and identified by 16S rDNA sequencing analysis. The isolates belonged to 6 different species of acid lactic bacteria (LAB) and *Staphylococcus epidermidis*. *Enterococcus faecium* was the most frequently isolated species (40.8%) and faeces (42.5%). According to the mother's lifestyle, we noticed that the genus *Enterococcus* was the most frequently isolated from rural mother's milk as well as urban mother's milk. *Lactobacillus fermentum* ($P \leq 0.05$) and *Staphylococcus epidermidis* ($P \leq 0.01$) were isolated only from rural mother's milk. An antimicrobial activity was observed in 30 strains from 148 LAB, the higher level of antagonist was with *E.faecium* (35 mm). The observed results showed that the isolated strains from rural mother's milk were different from that urban mother's milk. Eventual studies can be carried out about lifestyle and nutrition of mothers to explain the effect on the flora found in the milk and feces infants.**

**Keywords**: Human milk, Feces infant, Bacteria, identification, Antimicrobial activity.

Breastfeeding is largely recognized as an important factor for the child's health after birth. It is associated with lower infant morbidity and mortality, as well as with reduced incidence and severity of infections[1 2 3]. The establishment of the gut microbiota in newborns is a crucial step for gut maturation[4 5], metabolic and immunologic programming, and consequently health status[6].

Human milk appears as an important source of bacteria for the colonization of the infant's gut and the same species of bacteria are

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found in the infant's feces and their mother's milk[7 8 9 10 11]. The microbiome of human milk is very diverse. Sequencing of the 16S rRNA gene has shown the complexity of the bacterial community in breast-milk[12 13 14] , its variability among women and its relative stability over time[15 12]. Culture-independent methods consistently show *Staphylococcus*, *Pseudomonas*, and *Streptococcus* having the most relative abundance[16 12 17].

On the other hand, culture-dependent methods have shown the prevalence of the facultative anaerobic genera *Staphylococcus*, *Streptococcus* and *Propionibacterium*. Other genera isolated comprise *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Veilonella*, and *Rothia*[9 18 19 17 20]. The presence of lactic acid bacteria (LAB) in human milk is well documented[7 21 19].

Therefore, milk is a source of lactic bacteria for the infant gut [7 19]. LAB form a large group of anaerobic and aero-tolerant, non-sporulating, Gram-positive, catalase and oxidase-negative rods and cocci, generally with complex nutritional requirements. They produce antimicrobial substances, such as bacteriocins and organic acids, which can inhibit the growth of gastro intestinal pathogens in the infant's gut[22 23 24 25]. Bacteriocins have important potential applications in food and pharmaceutical industries[26 27]

The transmission of LAB with antibacterial activity through breast-feeding could play an important role in the prevention of intestinal infections and in the establishment of a healthy gut microbiome in the infant. The aim of our research was to identify the bacteria from mother-infant couples in Algeria. The second aim was to assess the antibacterial properties of LAB from breast milk and infants' feces. Few publications have addressed the question of the antimicrobial activity of LAB from breast-milk or infants, despite its potential contribution to their probiotic potential[28 29 30].

Moreover, previous studies on breast milk and infant gut microbiota have been conducted mostly in western countries or China. Yet there are geographical differences in infant's gut microbiota and environmental factors, such as diet and familial environment, are known to influence its composition[31 6]. In our work, we have screened mother-infant couples in Algeria, a country where few studies have been conducted in the field[32].

## MATERIAL AND METHODS

### Sampling and isolation of LAB

The 32 couples mothers-infant were recruited from the hospital "Etablissement Hospitalier Universitaire d'Oran, EHUO". The collected breast milk samples and feces were analyzed within the Service de Bactériologie, Etablissement Hospitalier Universitaire d'Oran (EHUO).

All participants were selected according to the following criteria: healthy women without current or past infection, full-term pregnancy and absence of infant or maternal perinatal problems. The infants were full-term vaginally delivered and breastfed. The mothers were informed of the procedures and the aims of the experiment, and gave their consent to the taking of samples. The milk samples were collected in sterile tubes, by manual expression using sterile gloves and after wiping the nipples and areola with a swab soaked in sterile water. The first drops of milk were not collected. Feces samples of the newborns (1g) were collected with sterile swab immersed, in 9 mL of sterile saline to allow the dispersion of samples.

Samples were collected from 32 mothers and their infants aged from 1 to 6 months. The samples were processed immediately after collection. Serial dilutions of the samples were plated in triplicate on specific culture media: de Man, Rogosa and Sharpe agar (MRS, Oxoid) supplemented with 0.5g/L of L-cysteine (MRS-cys) and Bifidobacteria Selective Medium agar (BSM)[33]. The plates were incubated at 37°C under anaerobic conditions (AnaeroGen, Oxoid) for 48 h to 72 h. Colonies with different morphologies were streaked on the appropriate medium. Purified isolates were tested for catalase and oxidase, and submitted to Gram staining. All isolates which were catalase-negative, oxidase negative and Gram-positive were kept for further analysis and stored at -80°C as frozen cultures in MRS broth with 50% glycerol.

### Identification of the isolates

An identification and biochemical characterization of some isolates was obtained

with the VITEK® 2 Advanced Colorimetry™ (BioMérieux). Cell suspensions and subsequent analyses were made according to the manufacturer's recommendations.

For the molecular identifications, an almost complete 16S rRNA gene fragment was amplified with the primers pH (AAGAGGTGATCCAGCCGCA) and 10-27Fd (a slightly shorter pA primer AGTTTGATCCTGGGTCAG)[34] or BifiFd (TYMTGGCTCAGGATGAAC). The latter primer corresponds to position 17 to 34, which is conserved in lactic acid bacteria and bifidobacteria, and was used when the initial amplification with the 10-27Fd primer failed. We used a standard PCR program of: 1 min. at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at 55°C and 2 min at 72°C, and ending by 10 min final elongation at 72°C in a T100 Thermocycler (BioRad). The amplification products were sequenced (Beckman Coulter Genomics, UK) with the primers used for the amplification.

A preliminary Blast analysis[35] of the sequences provided the genus to which each isolate belonged. The MEGA 5.2 software[36] was used to build phylogenetic trees from the multiple alignments with the type strains of the genus, using both the neighbour-joining method[37] and maximum likelihood. A taxonomic allocation was inferred from the closest reference strains the isolates clustered with. All sequences were deposited in GenBank under the access numbers KY552930 to KY552956.

**Assay of antibacterial activity**

Six commonly used indicator strains: *Escherichia coli* ATCC 25 923, *Pseudomonas aeruginosa* ATCC 43 300, *Staphylococcus aureus* ATCC 25 923, *Listeria innocua* ATCC 29 523, *Listeria ivanovii* ATCC 29 723 and *Micrococcus luteus* ATCC4698, were used for the detection of antimicrobial activity. They were cultivated in TSB (Tryptic Soy Broth, Difco) and 200 µl of the indicator culture were spread on to the surface of TSA (Tryptic Soy Agar, Difco) plates, to make indicator plates. Cell-free supernatants of the isolates to be tested were obtained by centrifugation of fully grown cultures at 13000 rpm for 5 min and filtration of the supernatant through a 0.45µm filter. The pH of all supernatants was adjusted to pH 6.5 with NaOH 1M.

Antibacterial activity was tested by the paper disc diffusion assay[38] and by the well-diffusion assay**.** In the first case, sterile paper discs (9 mm antibiotic assay paper discs) were placed on the surface of the indicator plate immediately after spreading and 100 µl of the cell-free supernatant of the isolates to be tested were deposited on the paper discs. The plates were incubated for 18 h to 24 h at 37°C. In the second case, wells were aseptically punctured into the indicator plates and filled with 100 µl of the cell-free supernatants. The plates were left at 4°C for 30 min and then incubated at 37°C for 24 h. The antimicrobial activity of the isolates was estimated from the size of the inhibition zone around the wells or the paper disc[39] .

**Proteolytic treatment**

A 20-µL volume of proteolytic enzymes (proteinase K, Thermofisher or trypsin) in their recommended buffers, and at a final concentration of 0.1mg/mL, was added to 200µL of supernatans (filtered and neutralized as stated before). The tubes were incubated at 37°C for 2 hours, and then puted at 100°C for 5 min to stop proteolysis.

**Acid and bile tolerance test**

Resistance to bile was tested as stated in[40 41]. In short, cells pretreated in PBS pH2.0 for 3 hours, were used to inoculate MRS medium with 0.2% and 3% bile (bovine bile, Sigma). Growth was assessed after 24h anaerobic incubation at 37° by plating serial dilution of the cultures.

**Statistical analysis**

The statistical analysis was performed with SPSS 20 software. The Pearson's tests were applied on different species of bacteria from breast milk and infant feces; urban and rural lifestyles of mothers. In the descriptive analysis, correlation coefficient was used for statistical analysis of this study. A *P-value* of $<0.05$ was considered to be statistically significant.

## RESULTS

**Diversity of lactic bacteria in breast milk and infant's feces**

Suitable dilutions of the milk and feces samples from the 32 mother-infant couples were spread on MRS-Cys and BSM agar plates. For each sample, colonies with different phenotypes were picked up and purified. A total of 155 isolates

(81 from milk and 74 from faeces samples) which were catalase-negative, oxidase-negative and Gram-positive were identified and kept for further analysis. Cocci (130 isolates) were identified by VITEK® 2 Advanced Colorimetry™. All isolates of this group were URE negative and grew in the presence of 6.5% of NaCl. They fell in 5 phenotypic groups (Table 1).

The identification by Vitek2 was later confirmed by sequencing the 16S rRNA gene of a few isolates of each group. All sequences were over 99% identical to the closest type strain.

BSM medium was expected to yield *Bifidobacterium* isolates. Yet none were identified among the isolates. Colonies growing on BSM medium were identified as *Lactobacillus fermentum*, *E. faecium*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*. *Staphylococcus epidermidis* colonies were also found on BSM medium.

Among 32 samples from mothers and infant (milk/infants feces) couples, we isolated and identified 7 species (Table 2), *Enterococcus feacium* (38,3% /43,24%, r=constant, *p* constant), *Enterococcus durans* (34.6% /21,62%, r=0.86, *p*=0.64), *Enterococcus feacalis* (9,87% / 20,27%, r=-0.168, p=0.555), *Lactobacillus fermentum* (4,93% /10.81%, r=0.218, p=0.23), *Staphylocoque epidermidis* (9,87% /1.35% , r=0.311, p=0.083). We noted that *Enterococcus feacium* was isolated in the majority of the milk and fecal samples.

A positive correlation is observed for 6 groups and negative for *Enterococcus feacalis*. For *Leuconostoc mesenteroides* and *Lactococcus lactis*, the correlation is significant at the level of 0.01 with (P-value= 0).

According to the mother's lifestyle, we noticed that the genus Enterococcus was the most frequently isolated from rural mother's milk as well as urban mother's milk, respectively : *E. faecium*

**Table 1.** Phenotypic identification of Enterococci by VITEK® 2 Advanced Colorimetry™

| Group | Phenotype | Number of isolates | Vitek2-based Identification |
|---|---|---|---|
| 1 | TyrA (+), AspA (+), AGAL (+), Sac (+), BGAL (+), ADH(+) | 50 | *E. faecium* |
| 2 | TyrA (-), AspA (-), AGAL (-), Sac (-), BGAL (-), ADH (+) | 10 | *E. faecium* |
| 3 | TyrA (-), AspA (-), AGAL (-), SAC (-), BGAL (-), ADH (-) | 3 | *E. faecium* |
| 4 | AMY(+), AspA (-), AGAL (-), Leu (+), ALaA, O129R (-), AGLU (+), dSOR (+) | 23 | *E. faecalis* |
| 5 | CEDEX (-), LAC (-), dMAN (-), AGAL (-), Sac (-), BGAL (-), ADH (-), ALaA (+), SAL (-) | 44 | *E. durans* |

**Table 2.** Comparison of frequencies of bacterial genera detected in breast milk and infant feces

| Species | Breast milk n = 32 | | Infant feces n= 32 | | Statistical analysis | |
|---|---|---|---|---|---|---|
| | N | % | N | % | Coef. correlation | *p*- value |
| *Lactobacillus fermentum* | 4 | 4.93 | 8 | 10.81 | 0.218 | 0.23 |
| *Enteroccoccu faecium* | 31 | 38,3 | 32 | 43,24 | Constant | constant |
| *Enteroccoccu durans* | 28 | 34,6 | 16 | 21,62 | 0,86 | 0,64 |
| *Enteroccoccu faecalis* | 8 | 9,87 | 15 | 20,27 | -0,168 | 0,555 |
| *Staphyloccoccus epidermidis* | 8 | 9,87 | 1 | 1,35 | 0,311 | 0,083 |
| *Leuconostoc mesenteroides* | 1 | 1,23 | 1 | 1,35 | 1** | |
| *Lactococcus lactis* | 1 | 1,23 | 1 | 1,35 | 1** | |
| Total | 81 | | 74 | | | |

N : total number of bacteria in %
n : sampling quantity
** : significant correlation at level 0.01 (bilateral)

(35.55% /41.66%, p=0.154), *E. durans* (28.88% /41.66%, p=0.325) and *E. faecalis* (4.44% /16.67%, p=0.325) (Figure 1 and Table 3).

For the following genera: *Lb. fermentum*, *S Epidermidis*, *Ln. mesenteroides*, and *Lactococcus lactis*, were isolated only from the rural mother's milk respectively: 8.88%, 17.77%, 2.22%, and 2.22%.

A significant correlation is observed for *Lactobacillus fermentum* (-0.378, $P \leq 0.05$) and *Staphylococcus epidermidis* (-0.577, $P \leq 0.01$). For other genera, *Enterococcus feacium*, *Enterococcus durans*, *Enterococcus feacalis, Leuconostoc. mesenteroides*, and *Lactococcus lactis*, none significant correlation is noticed.

**Antimicrobial activity**

All LAB isolates were tested for their antimicrobial activity against 6 indicator strains (*E. coli*, *L. innocua*, *L.ivanovii*, *M. luteus*, *P. aeruginosa*, and *S. aureus*). Thirty isolates (21% of all LAB isolates) had a marked inhibitory effect against at least one of the indicator strains in a plate assay. Isolates with antimicrobial activity were not distributed evenly between the different species (Table 4).

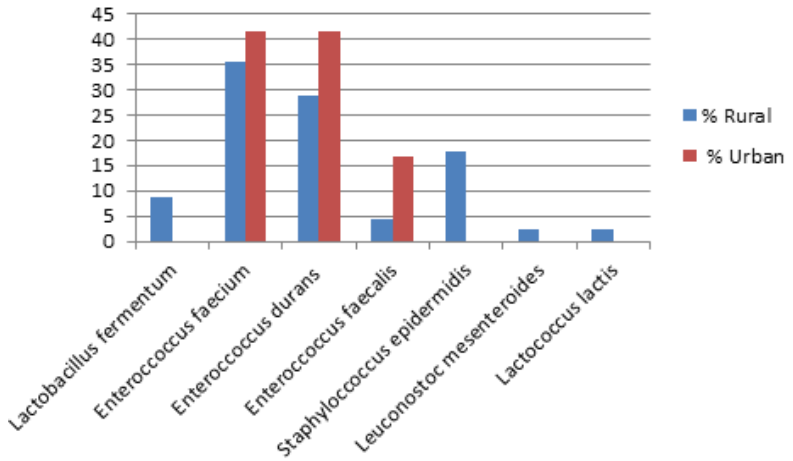


**Fig. 1.** Distribution of different isolates (species) of the milk mothers from the rural and the urban regions

**Table 3.** Comparison of frequencies of bacterial genera detected in mother's breast milk from rural and urban regions

| Species | Milk (n=16) Rural mother | | Milk (n=16) Urban mother | | Statistical analysis | |
|---|---|---|---|---|---|---|
| | N | % | N | % | Coef. correlation | *p* -value |
| *Lactobacillus fermentum* | 4 | 8,88 | 0 | 0 | -0,378* | 0,033 |
| *Enteroccoccus faecium* | 16 | 35,55 | 15 | 41,66 | 0,258 | 0,154 |
| *Enteroccoccu durans* | 13 | 28,88 | 15 | 41,66 | -0,18 | 0,325 |
| *Enteroccoccu faecalis* | 2 | 4,44 | 6 | 16,67 | 0,18 | 0,325 |
| *Staphyloccoccus epidermidis* | 8 | 17,77 | 0 | 0 | -0,577** | 0,001 |
| *Leuconostoc mesenteroides* | 1 | 2,22 | 0 | 0 | -0,180 | 0,325 |
| *Lactococcus lactis* | 1 | 2,22 | 0 | 0 | -0,180 | 0,325 |
| total | 45 | | 36 | | | |

N : total number of bacteria
n : sampling quantity
*: significant correlation at level 0.05 (bilateral)
** : significant correlation at level 0.01 (bilateral).

**Table 4.** Distribution per species of isolates with antibacterial activity

| Species | Number tested | Isolates with antimicrobial activity | Number of isolates inhibiting *P. aeruginosa* | *L. ivanovii* | *L. inoccua* | *M. luteus* |
|---|---|---|---|---|---|---|
| *Lb. fermentum* | 12 | 25% | 2 | none | none | 2 |
| *E.faecium* | 65 | 29% | 10 | 5 | 4 | 12 |
| *E.durans* | 40 | 0% | none | none | none | none |
| *E.faecalis* | 23 | 30% | 7 | 2 | none | 2 |
| *Lc.lactis* | 2 | 50% | 1 | none | none | - |
| *Ln.mesenteroides* | 2 | 0% | none | none | none | none |

**Table 5.** Probiotic and antagonism properties of the isolates with antimicrobial activity

| Isolate | Species | Sample type | Inhibition zone (diameter in mm) on indicator strains: *P. aeruginosa* | *M. luteus* | *L.innocua* | *L. ivanovii* | Growth in the presence of bile salt (2%)[1] |
|---|---|---|---|---|---|---|---|
| Dpt | *E. faecium* | Feces | - | - | - | 12 | - |
| D3 | *E. faecium* | Milk | 22 | - | 10 | - | + |
| D5 | *E. faecium* | Milk | - | 35 | - | - | + |
| D8 | *E. faecium* | Milk | 35 | 9 | - | - | + |
| D20 | *E. faecium* | Feces | - | 30 | 11 | - | + |
| D21 | *E. faecium* | Feces | 35 | 10 | - | 11 | + |
| D22 | *E. faecium* | Feces | - | 33 | - | - | - |
| D26 | *E. faecium* | Milk | 12 | 14 | 13 | 12 | + |
| D27 | *E. faecium* | Feces | - | 15 | 12 | 12 | - |
| D37 | *E. faecium* | Feces | - | - | - | 9 | + |
| M3 | *E. faecium* | Milk | - | 10 | - | - | + |
| M7 | *E. faecium* | Feces | 13 | 12 | - | - | - |
| M9 | *E. faecium* | Milk | - | 30 | - | - | + |
| M10 | *E. faecium* | Milk | 30 | 9 | - | - | + |
| M20 | *E. faecium* | Milk | 25 | - | - | - | + |
| M24 | *E. faecium* | Feces | 28 | 9 | - | - | + |
| M32 | *E. faecium* | Milk | 32 | - | - | - | + |
| D50 | *E. faecium* | Feces | 10 | - | - | - | - |
| Dgr | *E. faecalis* | Feces | - | - | - | 13 | - |
| R44 | *E. faecalis* | Milk | 15 | - | - | - | + |
| M15 | *E. faecalis* | Milk | 10 | - | - | - | + |
| M22 | *E. faecalis* | Milk | 29 | - | - | 12 | - |
| M29 | *E. faecalis* | Feces | 25 | 10 | - | - | + |
| D19 | *E. faecalis* | Milk | 9 | - | - | - | + |
| M4 | *E. faecalis* | Feces | 25 | 10 | 11 | - | + |
| D48 | *E. faecalis* | Feces | 26 | - | - | - | + |
| M28 | *Lc. lactis* | Milk | 33 | - | - | - | + |
| M1 | *Lb.fermentum* | Milk | - | 12 | - | - | + |
| M14 | *Lb.fermentum* | Feces | 15 | - | - | - | + |
| M25 | *Lb.fermentum* | Milk | 11 | 20 | - | - | + |

[1]:+: growth after treatment of 3h at pH3.0 in the presence of 2% bile salt; -: no growth after exposure to bile salts.

None of the 40 *E. durans* isolates (group 5 in Vitek2-identified *Enterococcus* isolates) had any antagonist activity against the indicator strains, and neither did the *Leuconostoc* isolates. Most of isolates with antibacterial activity belonged to the species *E. faecium.* Nine isolates were antagonist against more than one indicator strain (Table 5). Multi-antagonist isolates were found among *E. faecalis* (M4) and *E. faecium* (D21, D26 and D27).

Interestingly, none of the isolates had any effect on *E. coli* or *S. aureus*, but a high proportion (20 of the 30: 67%) of the antagonist isolates had an inhibitory effect on *Pseudomonas*. Isolates D8 *(E. faecium)* and M29 *(E. faecalis)* and the *Lactococcus* isolate had the highest antibacterial activity against *P. aeruginosa*. Large inhibition zones (of more than 30 mm) were also produced by four *E. faecium* isolates against *M. luteus* (Table 5).

The active supernatant retained their antibacterial activities after heat treatment (30 min at 100°). A treatment with proteinase K or trypsin results in the loss of the antimicrobial activity of all the active supernatants. This suggests that the antimicrobial activity is due to a heat stable, peptidic, bacteriocin-like product.

Most antagonist strains grew in the presence of bile salt (Table 5), an interesting capacity when looking for potential probiotic strains.

## DISCUSSION

Our study was done on a sampling of the Algerian population, 32 mothers-child couples resident in rural regions (n=16) and others in urban regions (n=16). Mother's maternal milk and feces of their babies aged from 1 and 6 months old.

We isolated and identify 81 bacterial strains from the maternal milk, 67 enterococci (82.71%), 8 staphylococci (9.87%), 4 lactobacilli (4.93%), 1 leuconostoc (1.23% and 1 lactococci (1.23%).

74 bacterial strains were identified in infant feces samples, 63 enterococci (85.13 %), 8 lactobacilli (10.81 %), 1 staphylococci (1.35 %), 1 leuconostoc (1.35 %) and 1 lactococci (1.35 %). We noted that the same genura are identified in the human milk as well as in the feces.

Most of the LAB isolated during this study belonged to the genus *Enterococcus*. This confirms what has been related in other studies were *Enterococcus* was predominant in breast-milk and infants feces[17].

Studies have indicated that Enterococci are the most commonly isolated species during the first two months of infancy, with Lactobacilli being the second most common early colonizer**[19].** Our results are also in accordance with previous studies of human milk based on a culture-dependent approach where *S. epidermidis* was one of the predominant species[19 17]. Staphylococcus appears also as a dominant genus through the 16S rRNA gene abundance in culture-independent approaches[16 17].

No significant difference (P- value > 0.05) was observed for four genera : *Lactobacillus fermentum, Enterococcus durans*, *Enterococcus feacalis, Staphylococcus epidermidis* isolated from the babie's feces and the maternal milk. This means that there is a homogeneous distribution of these genera in these two environments. Our results are in agreement with the previous studies which showed that the bacterial composition of the fecal microbiote of the breast-fed babies reflects that of the maternal milk [42]

Our data show the presence of lactobacilli (with 8.88 %) only in the maternal milk of resident mothers in rural areas[43] noticed that lactobacilli tends to have more important percentages in the milk of the resident women in rural areas. Our results are also in agreement with previous studies realized in South Africa, in Japan and South Korea[44]. Another Japanese study showed that rural women seem to have a higher prevalence of *L. reuteri* colonization than women from the other countries. This may be related to the wide use of functional foods, probiotics and various fermented foods as an important part of the Japanese diet[45].

In this study, the diet of lactating mothers was using a questionnaire that in rural maternal local bread, local fermented cheeses, raw goats' milk and druid fruit consumption was higher suggesting that the incorporation of probiotics in the mother's diet before delivery and in the infant diet during breastfeeding may positively influence the maturation process of gut immunity.

In our study, bifidobacteria was not identified as noticed in our previous works [46]. On the other hand, the majority of the papers published

previously show the presence of *Bifidobacterium*. This could reflect a diminished importance of members of this genus in the Algerian-mother-infant couples, due to the differences in nutrition, the way of life and genetics. All this factors influenced microbial composition of human milk[47].

We have identified in this study, the species *Enterococcus faecium*, *E. faecalis*, *Lactobacillus fermentum* and *Lactococcus lactis* as the most promising in terms of antagonism. Some recognized probiotic strains belonged to those species[48]. As we tested the antimicrobial activity on a limited range of commonly used indicator strains, it can be expected that a more extended survey, on a higher number of strains, would reveal a higher proportion of antagonists among the milk or feces isolates.

Our results suggest that the production of bacteriocins could explain the antimicrobial activity of the isolates. A conventional bacteriocin would not act on Gram-negative species. Yet, we have observed a high proportion of isolates with an antibacterial effect on the Gram-negative *P. aeruginosa*, a less frequent property of antagonistic LAB, although observed in some studies[49]. If the peptidic nature of the antagonist molecule was to be confirmed, its mechanism of action would be worth investigating.

Eight isolates (D21, D26, M7, M10, M24, M29, M4, M25) cumulate an antagonist effect against *P. aeruginosa* with an effect on the Gram-positif species of *Micrococcus* and *Listeria*. This could reflect the possibility that some LAB possess several antimicrobial mechanisms.

In this study we performed a screening of 30 antagonist strains. The advantages of these thirty particular isolates deserve investigation of a new in vivo study to determine their produced antimicrobial substances. Some of these strains could be exploited in the development of dairy formulas for babies or new food products. Bacteriocin-producing strains can also play an important role in food fermentation and preservation.

## CONCLUSION

This present research is the first study related to the microbiome of women breast-milk from different regions, done in Algeria. This work indicates that lifestyle of mothers (rural and urban) influences the presence of bacteria in their breast milk, which we have noted for the 32 milk samples of resident mothers from different regions. A significant difference is observed for two species, *Lactobacillus fermentum* and *Staphylococcus epidermidis*.

Our study confirms that human milk and infant feces analyzed in pairs share the same species, indicating that breastfeeding could contribute to the bacterial transfer from mother to child and thus colonize the intestinal flora of the baby.

To compare the flora of breast milk according to the lifestyle we suggest increasing the number of samples to confirm this result and collect accurate data on lifestyle such as diet, social conditions and economical life.

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