Probiotics and Mastitis During Lactation

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Abstract

Probiotics are defined as living microorganisms that confer a benefit for human health and their benefits are promising in lactating women. Recently it is known that human milk is not sterile and the microorganisms that have been isolated are particularly attractive because they perform anti-infective, anti-inflammatory, metabolic and immunomodulatory functions. By these benefits the bacteria of human milk have been considered as a target therapeutic in inflammatory processes associated with dysbiosis as Puerperal mastitis, the latter considered a cause of avoidable abandonment of human breastfeeding. Although the results of studies carried out on the treatment of mastitis during lactation with probiotics have had controversial results, they appear to be more encouraging in the prevention of this condition.

Keywords: Probiotics; Mastitis; Lactation

Probiotics and mastitis during lactation

Bacterial diversity of human milk

The milk traditionally considered sterile, but recently several studies have revealed that colostrum and milk are a continuous source of potentially probiotic bacteria for the lactated infant's intestine, as they consume approximately $1 \times 10^5$ and $1 \times 10^7$ bacteria per day. Mammary microbiota is peculiar and transient: its development begins during the last third of pregnancy, reaches the greatest complexity at the end of that period, remains quite constant throughout breastfeeding and decreases abruptly with weaning. The first descriptions of the bacterial diversity of milk in healthy women were based on the use of culture media and showed predominance of staphylococcus, streptococcus, lactic acid bacteria, Propionibacterias and Gram-positive bacteria, but it has also proven to be a source of Bifidobacterium, Lactobacillus, Staphylococcus and Enterococcus [1].

In the year 2011 Hunt KM, et al. the first study of microbiome focused on milk was published Human, characterizing bacterial communities present in milk samples from 16 healthy women and showed that bacterial milk communities are complex. Streptococcus, Staphylococcus and Serratia represented more than 5% of the bacterial communities, another eight genera represented 1% of the communities observed in all the samples. Among the hundreds of operative taxonomic units detected in each woman's milk, only 9 (Streptococcus, Staphylococcus, Serratia, Pseudomonas, Corynebacteria, Ralstonia, Propionibacterium, Sphingomonas and Bradyrhizobiaceae) were present in the samples of all women and accounted for about half of the observed microbial community, although their relative abundance varied greatly between the women, understanding that the milk bacterial community human may differ according to the individual and the state of health of women in lactation period [1,2].

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Origin of bacteria isolated in human milk

The origin of the bacteria presents in the milk human it has become a controversial subject in recent years. Traditionally, it was believed that milk housed bacteria that were simply the result of bacterial contamination of the mother’s skin or the oral cavity of the baby, but several studies have indicated that human milk contains its own microbiota. Infrared photography has shown that some degree of retrograde flow to the mammary ducts may occur during suction. Obviously, this reverse flow can provide an ideal route for the exchange of bacteria from the baby’s mouth to the mammary gland; but is also obvious that milk human can be a source of bacteria for the mouth of the Infant. These and other findings suggest that at least some of the bacteria present in the maternal intestine could reach the mammary gland through an endogenous pathway [1].

Although the pathway and the mechanisms that some bacteria could use to traverse the intestinal epithelium and reach the mammary gland and other places have not yet been elucidated, some works have offered a plausible scientific basis. It has been shown that dendritic cells can penetrate the intestinal epithelium to capture non-pathogenic bacteria directly from the intestinal lumen. This mechanism may not be exclusive to dendritic cells, as CD18 cells, including macrophages, have been shown to be essential for extraintestinal dissemination. Dendritic cells and intestinal macrophages can retain small numbers of live bacteria for several days in the mesenteric lymph nodes and propagate through lymphatic circulation (mucosa associated lymphoid system) to other places like the mammary glands (Whole-mammary pathway). In addition, it is known that during the lactation period, the colonization of the mammary gland by cells of the immune system is a selective process regulated by the hormones Lactogenes [1,3].

The skin can also be an important source of bacteria for human milk. In fact, many species of bacteria associated with the skin, namely, Staphylococcus and Corynebacterium, have been isolated from human milk of women very much. These bacteria may be relocated in the milk during the ejection, specifically from the nipple, areolas and glands of Montgomery, although it is worth noting that these species are also associated with the mucous surfaces of the body, including that of the tract gastrointestinal. Also, the bacteria of the couple could contribute to the bacterial populations in the milk, since it is known that the couples share the oral microbiota and of the skin. In other cases, the stool can also be a source of contamination in human milk when there are poor sanitary conditions. These processes are responsible for the abundance of bacteria in human milk, some of these pathways of bacterial transfer have the potential to contribute to the microbiota of human milk. It is now understood that human milk contains its own populations of bacteria that provide important health benefits not only of the mother but also for the infant [4].

Mastitis: Definition

A publication of the World Health Organization on mastitis refers to this as an inflammatory condition of the breast, which may be accompanied or not by infection and is usually associated with lactation, so it is also known as mastitis of the puerperal lactation or mastitis [5]. The Academy of Breastfeeding Medicine in its clinical protocol number four poses a clinical definition, referring to a sensitive, hot and swollen chest area associated with a temperature of 38.5°C or more, chills, flu-like pains and systemic diseases [6]. Other non-clinical definitions make mention of mastitis as a process characterized by bacterial dysbiosis in the mammary gland [7]. Finally, it can be concluded that mastitis is inflammation in the course of lactation of one or several lobes of the gland or not accompanied by infection and represents one of the main causes of avoidable cessation of breastfeeding human [8].

Analysis of the milk of women with mastitis: bacterial diversity

A one has not fully understood how or because the transition from the colonization to the infection occurs, although several factors have been identified that may predispose women to develop mastitis. These range from host factors such as genetics, the presence of polymorphisms and even blood groups, to bacterial factors, for example, virulence factors of bacteria present, to medical influences that include the use of antibiotics and the Generalized administration of iron to women during pregnancy [4].

It is then understood that mastitis is a multifactorial disease caused by a variety of pathogens and is characterized by an alteration in mammary microbiota. Jiménez, et al. They compared the metagenome of 20 milk samples (half of the women with mastitis, while the oth-
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er half was taken from healthy women. Among the predominant bacterial sequences were Proteobacteria, Firmicutes and Bacteroidetes. The healthy microbiome included Staphylococcus, Streptococcus, Bacteroides, Faecalibacterium, Ruminococcus, Lactobacillus and Propionibacterium. In contrast, Staphylococcus aureus clearly dominated the microbiome in the samples of women with acute mastitis, while increases in readings related to Staphylococcus epidermidis in the milk of those who suffered from subacute mastitis. Fungi, protozoa and some virus-related sequences were also identified. Finally, the authors conclude that, in cases of mastitis, the microbiome of human milk reflects a loss of bacterial diversity and an increase in sequences related to suspected etiologic agents [9].

Contrary to the above, Marín., et al. when analyzing 647 samples of human milk from women with mastitis found that the Staphylococcus epidermidis was the most isolated species (87.6%), the group of Streptococcus the second most common (68.6%), while Staphylococcus aureus it was detected only in 22.1%. These results highlight the important role of coagulase negative Staphylococci and Streptococcus as causative agents of human mastitis [10].

Treatment antibiotic of mastitis

When mastitis occurs, despite making every effort to prevent it, should be treated early and adequately. If treatment is delayed or incomplete, recovery is less satisfactory. There is an increased risk of breast abscess and recurrence. The fundamental principles of mastitis treatment are: support tips, effective milk removal, antibiotic treatment and symptomatic treatment [5].

Although there is no consensus, it is generally recommended to start antibiotic therapy if symptoms persist more than 24 - 48 hours or to severe or worsening symptoms despite having applied the general measures described and proper breast clearing. In severe cases, intravenous antibiotic therapy may be required. Empirical treatment: being the Staphylococcus aureus the most frequently implicated germ, the antibiotics indicated are: Cloxacillin (1st choice), a first generation cephalosporin (Cephalexin or cefadroxil, as effective as cloxacillin and more comfortable administration) or Amoxicillin-Clavulanic (inconvenient for microbial ecology by its very broad spectrum), which will remain 10 - 14 days. In case of allergy to beta-lactamases or in populations where there are more than 10% of isolates of Staphylococcus aureus Methicillin Resistant (SARM) or suspected mastitis by MRSA antibiotics indicated (empirically and then guided by antibiogram) are clindamycin, vancomycin, linezolid or Cotrimoxazole [8].

In the absence of microbiological analyses and antimicrobial susceptibility tests, antibiotic treatment usually depends on the choice of the physician and not on the scientific evidence, leading to increased antimicrobial resistance among mastitis-causing agents, this empirical practice of antibiotic choice may be a relevant risk factor for acute or subacute episodes to become chronic or recurrent due to the selection of antibiotic-resistant strains. The results of the Marín., et al. when analyzing the 647 samples of human milk from women with mastitis indicated a noticeable resistance of staphylococci to Benzylpenicillin and erythromycin with differences between species, also found a significant number of streptococci multidrug-resistant. This study showed that chloramphenicol, gentamicin, ofloxacin and ciprofloxacin were the most effective antibiotics against mastitis pathogens. The findings of this research are relevant to identify trends in antimicrobial resistance among mastitis pathogens and to implement a rational treatment for this disease based on microbiological analysis and the profile of sensitivity to the antibiotics [4,10].

Human milk bacteria as biotherapeutic agents

Bacteria that are originally isolated from human milk are particularly attractive organisms, as they would meet some of the main criteria generally recommended for probiotics, such as human origin, a history of safe intake and prolonged by a particularly sensitive population (infants and infants) and adaptation to mucous substrates. Among the bacteria isolated from human milk, species such as L. gasseri, L. salivarius, Lactobacillus reuteri, L. Fermentum Bifidobacterium breve. They are considered among those who have a potential probiotic. These bacteria seem to be adapted in a unique way to reside in the human digestive tract and to interact with us in symbiosis from the moment of birth. In addition, it has been shown that some of the strains isolated from this biological fluid perform Anti-infective, anti-inflammatory, immunomodulatory and metabolic functions, both in vitro and in vivo, including studies in human beings [1].

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**Probiotics and mastitis**

Probiotics are defined as living microorganisms that confer a benefit for the health of the host beyond the inherent basic nutrition, when it is administered in adequate quantities as a food component or as a preparation not food [11,12]. Taking into account the characteristics of Staphylococcus, including the multi resistance to antibiotics, the formation of biofilms and mechanisms for the evasion of the immune response, the development of new strategies for the treatment of mastitis based on human milk probiotics, as an alternative or adjunct to antibiotic therapy, appears to be attractive [1,7]. Probiotic trials for the treatment of mastitis in dairy cows have had mixed results, some successful and some not successful. At the local level, an intramammary infusion of *Lactobacillus lactis*, led to a great regulation of cytokines and chemokines (“a massive immune response”) or neutrophils capable of eliminating mastitis pathogens [13].

The Department of Nutrition, Bromatology and food Technology of the Complutense University of Madrid has developed two clinical trials on the use of probiotics in mastitis during lactation, both studies have been referenced by several authors in related topics between them L. Fernandez., et al. [1,7] and Angeliki Angelopoulou., et al. [4]; and his conclusions have been useful for decision-making in the use of probiotics in acute mastitis.

The first work was published in August 2008 by E. Jiménez., et al. [14], the study included 20 women with clinical symptoms of staphylococcal mastitis, the study highlights that all met the criteria of: redness and pain in the breasts, flu-like symptoms (including fever > 38.5°C), a count of Staphylococcus in milk greater than 4 log10 CFU/ml and a count of leukocytes in Milk exceeding 6 log10 CFU/ml. Participants had received chemotherapy (Cloxacillin, clindamycin, amoxicillin-clavulanic acid and/or erythromycin) for 2 to 4 weeks without improvement, although this treatment had been completed 2 weeks before the study was carried out. The volunteers were randomly allocated into two groups (probiotic n = 10 and Control n = 10) and neither the volunteers nor the investigators knew the assignment during the investigation. The probiotic group received for 30 days a capsule with 200 mg of a lyophilized probiotic containing 10 log10 CFU of *L. salivarius* CECT5713 and *L. gasseri* CECT5714 isolated from the breast milk of healthy women; And the pleasure group received a daily capsule containing 200 mg from Methylcellulose. Finally, the milk samples Human They were obtained at the beginning of the study (before the ingestion of the first capsule considered day 0) and at the end of the trial period (day 30).

Two important results assessed in this study were the count of Staphylococcus and lactobacilli in milk samples and the evolution of clinical symptoms. For the first result on day 0 the total Staphylococcus count in the breast milk of all women varied from 4.04 to 5.54 log10 UFC/ml, with similar average counts in both groups (Kruskal-Wallis p 0.006) and the Lactobacilli could not be detected in any of the samples. It is striking that using the specific PCR of the species and the sequencing of the ARNr 16S, the isolated staphylococcus of the milk of the subjects 1, 2, 5, 8, 12, 14, 19 and 20 were identified as *S. aureus*, while those present in the rest of the women were identified as *S. epidermidis*. In the Day 30, the average count of *Staphylococcus* in the group of Probiotics (2.96 log10 CFU/ml) was statistically inferior to that corresponding to the control group (4.79 log10 CFU/ml). On the other hand, clinical symptoms were evaluated weekly by a midwife. In the Day 7, symptoms improved significantly among women in the group of Probiotics and in the Day 14 No clinical signs of mastitis were observed; In contrast, the clinical signs persisted in the women of the control group during the entire study period. So the conclusion of this study is that *L. salivarius* CECT5713 and *L. gasseri* CECT5714, appear to be an effective alternative for the treatment of infectious mastitis during lactation.

The second clinical trial was published in May 2010 by Arroyo., et al. [15], in the I am a student Participated 352 women with symptoms The Mastitis, All met the criteria of the first trial and was registered in the ClinicalTrials.gov database (NCT00716183) [16]. Participants were randomized into 3 groups (2 groups of probiotics and 1 group of antibiotics) and neither the participants nor the investigators knew the allocation during the investigation. This study lasted 21 days and, during this period, the groups of probiotics A (n = 124) and B (n = 127) Daily consumed a capsule with 200 mg of a lyophilized probiotic containing log9 log10 CFU of *L. fermentum* CECT5716 or *L. salivarius* CECT5713. Women in the Antibiotic Group (group C, n=101) received antibiotic treatment prescribed in their primary care centers. Breast milk samples were obtained from las volunteers at the beginning (day 0) and at the End (day 21) of the study. The evolution of mastitis, all met the criteria of the first trial and was registered in the ClinicalTrials.gov database (NCT00716183) [16]. Participants were randomized into 3 groups (2 groups of probiotics and 1 group of antibiotics) and neither the participants nor the investigators knew the allocation during the investigation. This study lasted 21 days and, during this period, the groups of probiotics A (n = 124) and B (n = 127) Daily consumed a capsule with 200 mg of a lyophilized probiotic containing log9 log10 CFU of *L. fermentum* CECT5716 or *L. salivarius* CECT5713. Women in the Antibiotic Group (group C, n=101) received antibiotic treatment prescribed in their primary care centers. Breast milk samples were obtained from las volunteers at the beginning (day 0) and at the End (day 21) of the study. The evolution of mastitis were observed; In contrast, the clinical signs persisted in the women of the control group during the entire study period. So the conclusion of this study is that *L. salivarius* CECT5713 and *L. gasseri* CECT5714, appear to be an effective alternative for the treatment of infectious mastitis during lactation.

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the symptoms was assessed on days 0 and 21 by the midwives of their primary care centers. At both times, was asked to the participants to rate the pain of their Breasts from 0 (extremely painful) to 10 (pain-free).

The results assessed were equal to those reported in the first trial. The bacterial counts in the samples of human milk on Day 0 were very similar in the 3 groups and oscillated between 4.35 and 4.47 log10 CFU/ml. In this work the dominant species was also S. epidermidis (73%), followed by S. aureus (43%) and S. Mitis (30%); and Lactobacilli could not be detected in any sample. In day 21, differences were found in the total bacterial counts of the 3 groups (Kruskal-Wallis, p < 0.001). The average values of the total count of log10 bacteria in probiotic groups (2.61 and 2.33 log10 CFU/ml for groups A and B, respectively) were significantly lower (P < 0.001) than the corresponding value in the antibiotic group (3.28 log10 CFU/ml). Mean reductions of 1.74 and 2.15 log10 cycles were observed in the total bacteria count in groups A and B, respectively, while in the antibiotic group the Reduction was significantly lower (cycle of 1.10 log10). There were statistically significant differences in bacterial counts of each dominant bacterial species (S. epidermidis, S. aureus S. mitis). In the 3 groups at the end of the essay (Kruskal-Wallis, P < 0.001) and were always lower (P<0.001) in probiotic groups than in the antibiotic group. The largest reductions in bacterial counts were found in group B (L. salivarius).

The antibiotics prescribed for group C women were amoxicillin-clavulanic acid (38.6%), amoxicillin (22.8%), cotrimoxazole (18.8%), Cloxacillin (17.8%) and erythromycin (2%). The effectiveness of these antibiotics in reducing bacterial counts differed significantly (Kruskal-Wallis, P<0.001 for total bacteria and S. epidermidis, P = 0.005 for S. aureus and P=0.018 for S. mitis). Cotrimoxazole reduced the average bacterial count by 2.5 log10 cycles and was particularly effective against S. aureus. Amoxicillin-Clavulanic acid led to a reduction of 1.22 log10 cycles of the average bacterial count, while the efficacy of amoxicillin and Cloxacillin was lower. The Counts of the 2 women who received erythromycin did not change at the end of the study.

In the evolution of clinical symptoms, the mean score of breast pain reported by women was similar on day 0 in all 3 groups, ranging from 2.01 to 2.35. On day 21, the pain score in the breasts had improved in most of the participants, but 11 women (11%) of the antibiotic group did not report any changes or felt a little worse. There were statistically significant differences (Kruskal-Wallis, P < 0.001) between breast pain scores in probiotic groups (8.68 and 8.61) and the score of breast pain in the antibiotic group (5.81) on day 21. Clinical symptoms disappeared or improved notably among most women allocated to any probiotic group, while evolution was variable among those assigned to the antibiotic group. In fact, all women (n = 9) who decided to stop breastfeeding during the trial belonged to the antibiotic group. The rate of mastitis recurrence in the antibiotic group (30.7%) was significantly higher than the corresponding rate in probiotic groups (x²=27.08, P < 0.001), but there was no difference between the groups of probiotics with respect to this parameter (rate for group A, 10.5% and rate for group B, 7.1%; x²=0.91, P < 0.340). Finally, this study also concludes that the use of probiotics (L. fermentum CECT5716 o L. salivarius CECT5713) It appears to be an effective alternative to the use of commonly prescribed antibiotics for the treatment of infectious mastitis during lactation.

Considering if the probiotics could prevent the episodes of mastitis, the same group of the Department of Nutrition, Bromatología and food Technology of the Complutense University of Madrid, published in November 2015 a clinical trial evaluating The prevention of mastitis using Lactobacillus salivarius PS2 in women at the end of pregnancy with antecedents of mastitis during lactation [17]. A total of 108 pregnant women, aged between 24 - 35 and 27 - 32 weeks of gestation, participated in this study. All met the following criteria: normal pregnancy, healthy status and a history of lactation mastitis after at least one previous pregnancy. The study was recorded in the ClinicalTrials.gov database (NCT01505361) [18]. The study was designed as a randomized, placebo-controlled, double-blind trial. The volunteers were randomized, in a proportion of 1:1, in 2 groups (“Probiotic” and “placebo”). The participants in the probiotic group (n = 55) they consumed a daily capsule with ~ 50 mg of lyophilized probiotic (~ 9 log10 CFU of L. salivarius PS2) from approximately 30 weeks of pregnancy to birth. Two dropouts were recorded in this group by factors related to the study.

“Acute mastitis” was defined as an acute inflammation of the Chest with local symptoms (engorgement, pain, redness, reduced milk secretion) and systemic (flu-like) symptoms, while “subacute mastitis” was defined as an inflammation of the Chest Accompanied by

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Discussion

Several strains of Lactobacilli have been isolated from human milk (L. salivarius, L. gasseri, L. fermentum) and it is believed that the presence of these microorganisms protects against mastitis. In the cows have been conducted trials with probiotics and the results are not conclusive, some cases are successful and others are not. In women several studies have been published by the same research center (Department of Nutrition, Bromatology and food Technology of the Complutense University of Madrid) two evaluating to treat mastitis and one to prevent it. But to these studies can be done some methodological criticisms that put into question their results and conclusions.

In the work done by E. Jiménez., et al. [14] the women included in the study had already been managed Antibiotic Cloxacillin, Clindamycin, amoxicillin-clavulanic acid and/or erythromycin for 2 to 4 weeks without improvement; But it is important to emphasize that some of the antibiotics that were used are not considered first line in the treatment of mastitis and treatment time exceeds the proposed 7 - 14 days, a situation that may predispose to Candida infections and Both consider non-improvement with antibiotic treatment that had ended at least 2 weeks before the study was carried out. The characteristics of the study correspond to that of a clinical trial by randomization and double blinding, although this was not described in the study and was not recorded in a clinical trial database. Although pain is a subjective perception, in this work there is no description of the use of similar pain scales by midwives evaluating weekly participants; By not having a measuring instrument there may be biases on the part of the observer by not objectively reflecting the variable.

The second clinical trial published by Arroyo., et al. [15] it also has some critical those that have been proposed by Amir; Lisa H and Al [13]. In an article titled “probiotics and mastitis: Evidence-based marketing?” the clinical trial published describes the results on day 21 and concludes that the women who received Lactobacillus fermentum or Lactobacillus salivarius had less pain and risk of mastitis recurrence than women who received antibiotic. However, the clinical trial database indicates that the data were collected on days 7, 14 and 28, not on day 21 [16]; However, only data from a tracking time point (“Day 21”) are reported in the published document. Anyone suffering from mastitis would expect an improvement on day 7, not three weeks later, why was this information omitted? In addition, although the document describes the trial as a blind randomised controlled trial, the clinical trial database information explains that the assignment
was “non-random” and that the physicians themselves prescribed antibiotics; therefore, women and clinicians were not blinded to the assign the treatment. The women received a variety of antibiotics: amoxicillin, amoxicillin-clavulanic acid, cotrimoxazole, erythromycin and Cloxacillin and many of them are recognized as inadequate for the treatment of mastitis, because *S. aureus* is commonly resistant to some of these antibiotics (amoxicillin, Co-trimoxazole, erythromycin). As in the previous study, bad results after inappropriate antibiotics are not surprising. In the follow-up, the reported symptom is “pain in the breasts”, which is only one of the mastitis indicators (the triad includes lumps and redness, as well as fever or “flu symptoms”). In the trial, women who received antibiotics were more likely to report pain in the breasts on day 21, but some of these women may have had symptoms of Candida infection, a common sequela of antibiotics in breastfeeding women. Amir Lisa H., et al. manifests that there are many problems with the essay conducted by Arroyo, et al.

In the case of the clinical trial taking into account the use of probiotics to prevent mastitis, using the strain *Lactobacillus salivarus* PS2 [17] there are also inconsistencies, the article reports that mastitis symptoms were assessed by midwives during the first 3 months after birth but The Clinical Trials Register [18] establishes that the primary result of this clinical trial it is: “Evidence of clinical mastitis confirmed by microbiological cultures and counts of Cells [Time frame: weekly during the first 6 months after birth]”, however, authors report data up to three months in the publication. The authors classified the episodes of mastitis as “acute mastitis” (signs and systemic symptoms) and “subacute mastitis” (only inflammation of the sinuses). The terms “acute” and “subacute” mastitis seem to be taken from research on bovine mastitis and are seldom used to describe the mastitis of human lactation. Physicians evaluating a woman with local mammary inflammation would diagnose this as a blocked duct instead of mastitis and would rarely prescribe an antibiotic [13].

The information available in the international trials registers indicates that there are three other ongoing trials to assess the effectiveness of two Lactobacillus strains in the prevention of mastitis in lactating women, who impress on better quality Methodological in your design [19-21].

Finally, there is not solid evidence to support the use of probiotics in mastitis. High-quality randomised controlled trials are needed to assess the effectiveness of probiotics for the prevention and treatment of mastitis.

**Conclusion**

Postpartum mastitis is an inflammatory disease of the breast glandular associated with bacterial dysbiosis and is considered one of the main causes of breastfeeding suspension. Probiotics from human milk, as an alternative or supplement to antibiotic therapy, appear to be attractive, although high-quality randomized controlled clinical trials are needed to assess their effectiveness. For now the only useful strategy is prevention based on educating the mother on an appropriate breastfeeding technique from pregnancy, for this reason we require a medical staff with updated breastfeeding knowledge and counseling skills.

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