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The Amendment of Antibacterial Properties and Bacterial Abundance of Breast Milk in Mothers with Hypothyroidism

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Abstract

Breast milk is the specific bio-fluid which nourishes infants by its special composition of nutrients, proteins, lipids, carbohydrates, biologically active components, and bioactive components. These components protect against infectious pathogens and develop the neonatal immune system. The long time ago, people believed that breast milk was sterile. But breast milk harbours different microbial communities which offer antibacterial properties against some human pathogens. As we have the doubt whether the hypothyroidism controls the probiotic bacteria of the victims, here, we tested 12 milk samples of healthy and thyroid (6+6=12) breast milk for their bacterial count and antibacterial properties by agar well diffusion method. Here, some paediatric pathogens like *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Streptococcus pneumonia* collected from infant's stool were tested to find their susceptibilities by the antibacterial activity of the breast milk. Results had shown that low bacterial count and lower antibacterial properties in the breast milk samples of people with hypothyroidism when compared to that of healthy people ($P < 0.01$). Finally, we found that *Staphylococcus aureus* is more susceptible among the two other pathogens. The present results emphasize the variation in both the bacterial count and antibacterial properties of breast milk bacterial communities in healthy and people with hypothyroidism. Thus, this research paper delineates the protective role of breast milk against various paediatric diseases and their perspective consequences both in healthy and people with hypothyroidism.

Keywords
Breast milk Bacteria, Antibacterial activity, Hypothyroidism, Bacterial count.

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1. INTRODUCTION

The human body contains 100 trillion cells. But, surprisingly one-tenth part of these cells is hardly real human cells. As the human body harbour trillions of bacteria, viruses, fungi, and other tiny organisms, collectively called as the microbiome, each type of organism plays a different role which reveals that human microbiome of different persons is not considered to be the same (1). Later scientists discovered the complexity of breast milk by its diverse microbiome and stem cells (3). A while back, people considered that breast milk was contaminated when found any type of bacterium (3). But, different studies disclosed that breast milk contains diversified bacteria like *Corynebacteria,* lactic acid bacteria*, Propionibacteria, Bifidobacteria, Staphylococci,* and *Streptococci* (4). Later, culture-independent techniques proved the presence of different bacteria in breast milk (5, 6, 7, 8, 9). In recent times, there are studies which showed the transfer of bacterial communities from mother to child through the process of breastfeeding (9, 10, 11). So, breast milk is the ideal nutritional formula for the infants during the first 6 months to one year of age. As milk contains a distinct array of bacteria and numerous biologically active components, it directs the initial mucosal immune system of infants (12).

There are some components in the breast milk which arise the growth of various bacterial communities in the gut of the infant (13). Some scientists declared that the origin of breast milk bacteria might be colonized with influence of the maternal skin (coagulase-negative *Staphylococci*, since *Staphylococcus epidermidis* as the predominant species) or the infant mouth (rarely found bacteria such as viridans *Streptococci* (14) or the cells of the mucosal-associated lymphoid tissue (MALT) (since the dendritic cells can pierce through the epithelium of gut to obtain the bacteria) (15).

Thyroid diseases are widespread worldwide. The considerable burden of thyroid diseases is increasing even in India. Based on the insights of various studies, 42 million people suffer from thyroid diseases in India in 2011 (16). The Indian Express published on July 17th, 2018 showed that over 30% of Indians suffer from thyroid diseases in India like thyroid nodules, hyperthyroidism, goitre, thyroiditis, and thyroid cancer.

2. METHODS

We surveyed for lactating women, especially people with hypothyroidism in Tirupati, South India, and its rural places. I got to know that those thyroid women are strenuous to conceive and give birth to the baby. So, we got 6 hypothyroid lactating mothers and 6 healthy mothers for control (age: 20-30 years). Some people from a rural background were not willing to give their breast milk due to shy and their culture. All women had healthy natural diet habits without any fast food intake. Breast milk samples were collected from 2 – 6 months postpartum (n=12). The collection of breast milk was achieved in the presence of a trained nurse in hygienic conditions after taking bath. We got written informed consent from the people who were willingly participating in this study and the present research was approved by the Institutional Ethical Committee, S V Medical College, Tirupati, India (Annexure No.1) and the samples collected from Government Maternity Hospital, Tirupati.

2.1 Collection of Breast Milk Samples:

Breast milk can be collected in different ways besides the hand expression with the help of the hospital grade electric pump. Here, we used both the normal sterile pumps and hand expression according to convenience. Breast milk samples were collected from mothers in sterile containers by manual expression using sterile gloves and sometimes with sterile breast pumps to get the good flow of the milk. First, the nipples of all mothers were cleansed with sterile water and soap water and soaked in chlorhexidine (Dr. Reddy's brands, India). Then, we divided the breast into 4 quarters and 4-cm^2 area of the outer upper quarter of the breast was cleaned with sterile cotton swabs which were previously soaked in ST solution (0.15M NaCl with 0.1% Tween 20) (17). Approximately 250 µl of the milk which was coming from the first time was discarded and then 10 – 15ml breast milk was collected. All these samples were kept at 4^{0}C (18) and later at -60^{o}C for further use. All the milk samples were separated from the mother's somatic cells with the help of centrifugation at 500g x g for 5 mins (19). Here, the people selected for the sample collection have not received any type of antibiotics and hadn't suffered from mastitis ever. The informed consent was obtained before the sample collection.

2.2 Breast milk bacterial count:

The plate count method was employed for counting of bacteria. We made milk samples into 10 dilutions (10^{-1} – 10^{-4}) with sterile distilled water. All the dilutions were spread over the Petri plates having Regosa and Sharpe (MRS) media to observe the presence of bacterial communities in CFU/ml.

2.3 Monitoring the antibacterial activity of breast milk samples against different bacteria:

Nutrient Agar Media (Hi-Media) was prepared in a sterile conical flask under aseptic conditions. Then it

was subjected to the autoclave at 121°C in 15 lbs pressure for 15 minutes. After sterilization, the media was poured into Petri plates and then left for solidification. Later, the wells were punched under sterile conditions. Selected bacterial cultures (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*.) kept overnight were added into Petri plates, and they were spread by using a spreader. The milk was just boiled for a few minutes (20). Then milk samples were added to wells at different concentrations. These Petri plates were wrapped by using the clean wrapper incubated at 37°C±2 °C for 24-48 hours. After 24 hours incubation, milk samples showed the zone of inhibition against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. Final pH was adjusted (at 25°C) to 7.4±0.2 with the help of 0.1N NaOH or 0.1N HCl.

Staphylococcus aureus which causes *Staphylococcal* Scalded Skin Syndrome (SSSS) and shows folliculitis, impetigo, boils on infant's skin, *Streptococcus pneumonia* producing pediatric respiratory infections and *Pseudomonas aeruginosa* affecting immune defences and urinary tract. Here, we were selected to test their susceptibilities against the capability of breast milk's antibacterial activity. These pathogens were isolated from the stool samples of infants.

2.4 Statistical analysis

Results were employed by One-way ANOVA to verify the significance ($p < 0.01$) of the variation happened in the antibacterial activities of breast milk in both healthy and people with hypothyroidism.

3. RESULTS

Bacterial count was observed in both healthy and thyroid breast milk samples. No growth of any prospective pathogens but the probiotic bacteria. The number of bacteria from healthy to people with hypothyroidism was reduced throughout the milk dilutions. For the first time, it was recorded that the count was reduced in the thyroid breast milk samples than that of healthy people. Finally, we have noticed no bacteria found at 10^{-4} & and 10^{-3} & 10^{-4} dilutions corresponding to healthy and thyroid samples.

Table: 1. Bacterial counts with different dilutions of healthy breast milk [cfu/ml]

Sample name	CFU/ml			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
H1	$3.25x10^2$	$0.11 x10^2$	$0.005 x10^2$	Nil
H2	$3.40 x10^2$	$0.19 x10^2$	$0.095 x10^2$	Nil
H3	$3.33 x10^2$	$0.13 x10^2$	$0.0045 x10^2$	Nil
H4	$3.15 x10^2$	$0.15 x10^2$	$0.005 x10^2$	Nil
H5	$3.23 x10^2$	$0.12 x10^2$	$0.0043 x10^2$	Nil
H6	$3.16 x10^2$	$0.14 x10^2$	$0.006 x10^2$	Nil

H1, H2, H3, H4, H5, H6: Healthy breast milk samples 1,2,3,4,5,6; cfu= Colony Forming Unit; 10^{-1},10^{-2},10^{-3},10^{-4}: Breast milk serial dilutions with distilled water.

Table: 2: Bacterial counts with different dilutions of thyroid breast milk [cfu/ml]

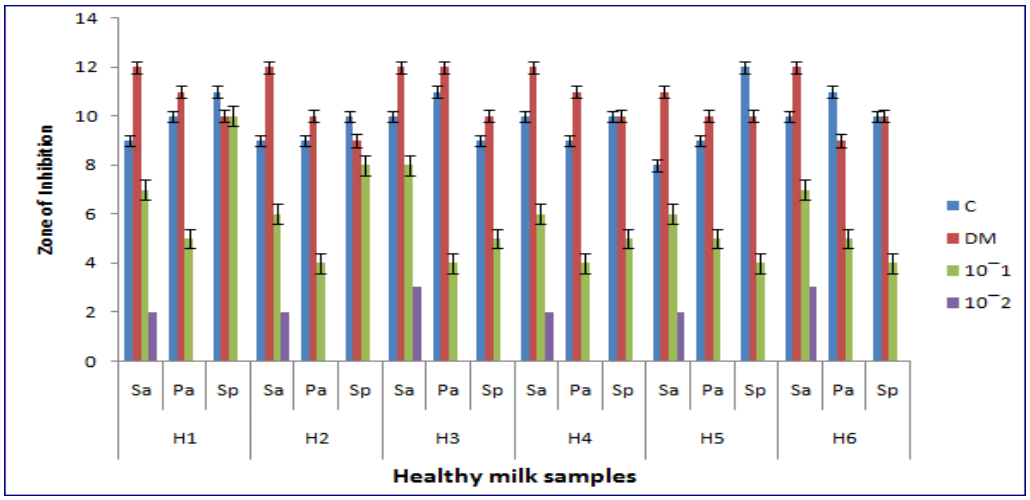
Sample name	CFU/ml			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
T1	$1.95 x10^2$	$0.095 x10^2$	Nil	Nil
T2	$1.01 x10^2$	$0.007 x10^2$	Nil	Nil
T3	$1.21 x10^2$	$0.089 x10^2$	Nil	Nil
T4	$1.90 x10^2$	$0.080 x10^2$	Nil	Nil
T5	$1.0 x10^2$	$0.005 x10^2$	Nil	Nil
T6	$1.05 x10^2$	$0.011 x10^2$	Nil	Nil

T1, T2, T3, T4, T5, T6: Thyroid breast milk samples 1,2,3,4,5,6; cfu= Colony Forming Unit; 10^{-1},10^{-2},10^{-3},10^{-4}: Breast milk serial dilutions with distilled water.

3.1 Antibacterial activity of healthy and thyroid breast milk samples with different dilutions against different pathogens

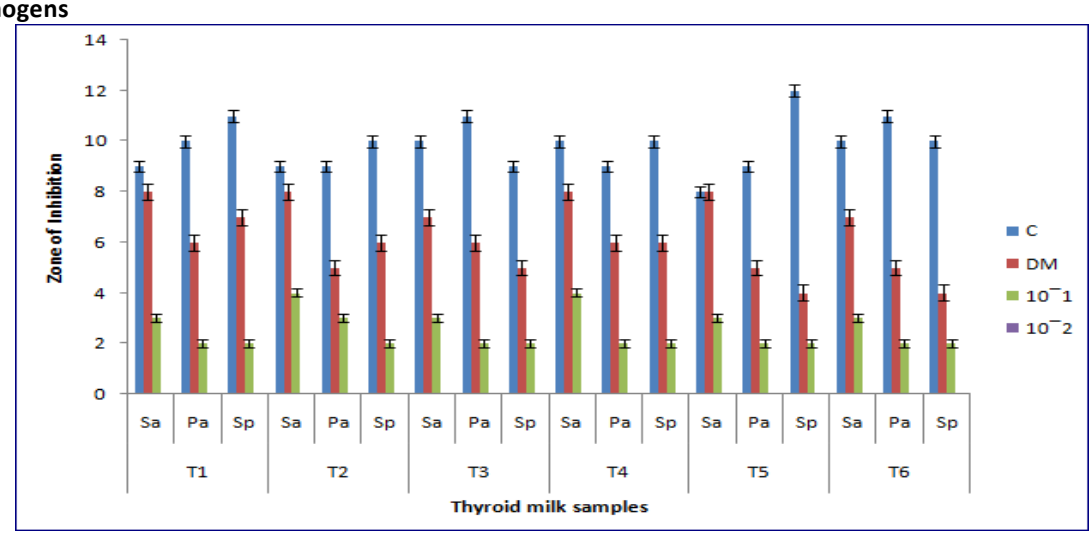
Antibacterial activity of healthy and thyroid breast milk samples was observed. Here, we used direct milk after just boiling and their dilutions with sterile distilled water (10^{-1} -10^{-2}). Inhibition zones formed by the standard antibiotics Tetracycline, Azithromycin, Ciprofloxacin were used as positive controls against *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa* respectively. The antibacterial properties of healthy breast milk were found to be greater than that of thyroid breast milk in their dilutions.

Figure: 1. Antibacterial activity of healthy breast milk samples with different dilutions against different pathogens



DM: Direct milk sample devoid of somatic cells; 10^{-1}, 10^{-2}: milk serial dilution with sterile water; H1, H2, H3, H4, H5, H6: Healthy breast milk samples 1,2,3,4,5,6; SA: *Staphylococcus aureus;* PA: *Pseudomonas aeruginosa*; SP: *Streptococcus pneumonia*; C: Controls: against SA is Tetracycline, against PA is Ciprofloxacin, against SP is Azithromycin.
(Results were expressed as mean with standard error indicated by vertical error bars from independent triplicates and the significant difference (P < 0.01)).

Figure: 2. Antibacterial activity of thyroid breast milk samples with different dilutions against different pathogens



DM: Direct milk devoid of somatic cells; 10^{-1}, 10^{-2}: milk serial dilution with sterile water; T1, T2, T3, T4, T5, T6: Thyroid breast milk samples 1,2,3,4,5,6; SA: *Staphylococcus aureus;* PA: *Pseudomonas aeruginosa*; SP: *Streptococcus pneumonia*; C: Controls: against SA is Tetracycline, against PA is Ciprofloxacin, against SP is Azithromycin.
(Results were expressed as mean with standard error indicated by vertical error bars from independent triplicates and the significant difference (P < 0.01))

4. DISCUSSION

Breast milk is a highly nutritious food for infants. It is the main source to feed infants and fulfil their daily nutritional requirements, furthermore, infants need protective agents against all pediatric diseases. Consequently, this study accentuates the defensive role of breast milk to build an infant's health by its antibacterial properties. Present results indicating the role of breast milk samples against the growth of pediatric pathogens like *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Streptococcus pneumonia.* It was shown that breast milk is able to control those pathogens than their respective standard antibiotics. Both the bacterial count and antibacterial properties were reduced with the dilution of the breast milk; besides that, thyroid

breast milk showed lesser activities in respect of healthy breast milk samples. These results were supported by Adegoke, (21) on antibacterial activities of direct breast milk and its dilution showed antibacterial activity against Diarrheagenic *Escherichia coli* (DEC) and they found antibacterial activity was reduced in diluted breast milk samples. For our knowledge, there were other studies that focussed on protective effect of breast milk against various pathogens (19, 22, 23, 24, 25, 26, 27, 28, 29) this is the first study gives an idea about the antibacterial properties of breast milk in thyroid and healthy people. Although there may be several reasons, further research has to be focused on the reasons why the thyroid breast milk bacterial count was reduced than that of healthy ones.

Thus, this study enlightens that breast milk can diminish the incidence of pediatric diseases by offering a broad collection of various superfluous defensive agents through redundancy.

5. CONCLUSION AND FUTURE PROSPECTIVES

Growth response of various bacteria against healthy and thyroid breast milk samples was tested and showed subtle changes in thyroid milk samples *i.e.,* reduced antibacterial properties in hypothyroid ones. Almost all the breast milk of the people with hypothyroidism had shown less antibacterial activity when compared to healthy breast milk. Among the 3 pathogens, *Staphylococcus aureus* had shown the higher susceptibility, later *Pseudomonas* and *Streptococcus.* As well as the bacterial count was also lessened in breast milk of people with hypothyroidism, it should be needed to scan all the bacterial communities present in both the breast milk sample during the extension of research with the help of Next Generation Sequencing (NGS) technology.

This study highlights the subtle changes *i.e.,* reduction of both bacterial count and antibacterial properties of breast milk belonging to the people with hypothyroidism. Nevertheless, further research has to be necessitated to find out the underlying cause in order to improve breast milk nutrition and probiotic microorganisms of people with hypothyroidism, alleviation of breast milk against various pathogens in hormonal imbalance conditions of lactating women (eg. People with hypothyroidism *etc.,*) is highly required to give the best nutrition to their new-borns to minimize the chaotic effects come to pass in infants.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this original research paper.

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