

# Determination of In-Vitro Antibacterial Effects of Breast Milk.

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## SUMMARY

**Background:** Breast milk undisputedly is the ideal baby food. It provides a lot of protective functions for the baby as well complete nutrition. It contains fat, proteins carbohydrates, vitamins and minerals. There have been various claims that it cures ailments. The study was therefore designed to substantiate those claims.

**Objective:** To determine the in vitro antibacterial effects of breast milk.

**Methodology:** Expressed breast milk of nursing mothers who were not on antibiotics was challenged with 0.5 McFarland's standard of overnight cultures of common bacteria that cause infections. The mixture was sub cultured at 30 minutes, 1 hour and lastly 2 hour intervals. The plates were read the following day for evidence of growth.

**Result:** Overnight incubation yielded growth of the various organisms that were inoculated.

**Conclusion:** Breast milk does not have *in vitro* antibacterial effect although it may possess same *in vivo* with the synergistic effect of other substances in the body.

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**Key words:** *in vitro*, antibacterial effects, breast milk.

## INTRODUCTION

Human milk is considered the ideal food for the new born during the first six months of life<sup>1</sup>. It is specifically adapted to the needs of the newborn infant<sup>2-5</sup>. It contains, in addition, a great array of 'bioactive factors' which provide the infant protection from infection by various micro-organisms<sup>6,7</sup>, hormones, and growth factors that affect development, agents that modulate immune function<sup>8</sup> as well as anti inflammatory components<sup>9</sup>.

Protection against acute infections of the digestive and respiratory tract of breast fed infants were reported about two decades ago<sup>10, 11</sup>. Then in the 90's carefully designed studies

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confirmed without any doubt that breast feeding protects the newborn from diarrhea, respiratory tract infections and otitis media<sup>12</sup> and reduces incidence of necrotizing enterocolitis<sup>13</sup>.

Human milk contains anti-inflammatory agents such as antiproteases, antioxidants and enzymes that degrade inflammatory mediators and modulators of leukocyte activation<sup>14</sup>. It is said that colostrums, secreted during the first few days after parturition, contains higher concentrations of proteins (including higher levels of protective proteins such as secretory IgA, Lactoferrin, and Lysozyme)<sup>15</sup>. There is paucity of information on in vitro antibacterial effects of breast milk in Nigeria. The study was done to determine the antibacterial effects of breast milk on some common gram positive and gram negative organisms.

## MATERIALS AND METHOD

**Study group:** Thirty nine (39) breast feeding mothers who had a spontaneous vaginal delivery at term in Nnamdi Azikiwe University Teaching Hospital, Nnewi were used in the study.

**Ethical issues:** The research posed no health hazard to the patients and was at no extra cost to them. Participation was voluntary and participants were free to withdraw at any point in the study. Confidentiality of the patients was maintained. Ethical clearance was obtained from the Research and Ethics Committee of NAUTH.

## METHODOLOGY

The research was accompanied with a questionnaire, which was filled by the mother or the doctor with information from the mother. The questionnaire contained demographic information, and knowledge of and use of breast milk for the treatment of ailments.

**Specimen collection:** 2-3mls of breast milk was expressed directly into a sterile container. Samples were transported immediately to the laboratory for processing.

**Test organisms include:** *Staphylococcus aureus*, *Klebsiella spp*, *Escherichia coli* and *Pseudomonas aeruginosa*,

**Control organisms include:** *Staph aureus* ATCC 29213 and *Escherichia coli* ATCC 25922.

## Laboratory procedures:

Overnight cultures of test organisms and control organisms were ready prior to sample arrival in the laboratory. As soon as specimens arrived, 2-3 drops of each specimen was distributed in 7 dry sterile Petri dishes. To each was then added 1 drop of either the test organisms or control strains standardized with 0.5 McFarlands standard. These were mixed very well. The

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mixture was sub cultured unto nutrient agar plates after 30 minutes, 1 hour and then finally 2 hours later. These plates were incubated overnight then read the following day.

In the second method, overnight cultures of the organisms/controls were plated out on agar plates. Then a sterile cork borer was used to make holes about 5mm diameter in the agar without puncturing the Petri dishes. Breast milk was dropped in the holes and this was incubated overnight as in the first method. The plates were examined the next day for zones of inhibition.

### RESULT

From the Questionnaire, 38 mothers (97%) said that they had heard about the use of breast milk for the treatment of ailments. Their source of information is seen in Table 1. Thirty three (84.6%) actually claimed they had used breast milk for treatment. Ailments used include conjunctivitis- 30 people (76.9%), boils- 9 (23.1%) and umbilicus- 4 people (10.3%).

After 24 hours incubation period, there was luxuriant growth of the organisms on all the inoculated agar plates in both the first and second methods. There was no recognizable difference in the degree of growth of bacteria seen after 30 minutes, one hour and two hours contact times respectively in the first method. In the second method, where a cork borer was used to make holes in the agar plates and breast milk was introduced into the holes, after 24 hours of incubation, the inoculated organisms grew luxuriantly all over the agar plate. There was no zone of inhibition at all.

**Table 1: Source of information about the use of breast milk for treatment of ailments.**

Source	Response (%)
Mother	30 (94.4%)
Aunt	20 (51.3%)
Friend	17 (43.6%)
Nurse	05 (12.8%)
Doctor	-

### DISCUSSION

Human breast milk is the ideal food for the new born during the first six months of life. It offers babies complete nutrition, early protection against illness, safe and healthy food. It contains fat, proteins, carbohydrates, vitamins and minerals. Under normal circumstances, freshly expressed human milk has a mild, slightly sweet scent and may contain microbial non-pathogens of skin flora<sup>16</sup>. The study was done to ascertain if breast milk had in-vitro anti bacterial effects on some common bacteria. It could be seen that there does not seem to be any anti bacterial activity in-vitro as the bacteria grew luxuriantly on all the plates that were incubated with no zones of inhibition.

It is also worthy of note that from the questionnaire, no medical doctor was said to have told the mothers about the topical use of breast milk in treatment of ailments. This could just be one of those myths being handed down from one generation to another without scientific basis. This does not in any way dispute the protection from the digestive as well as acute respiratory tract infections.

Studies have shown that protection from infection is provided by two main mechanisms classical immune protection provided by Immunoglobulins A, G and M in human milk<sup>12</sup>, as well as by a large array of other milk components that act as ligands for bacteria and viruses, and factors that fine tune the interaction among these agents which may also enhance the maturation of the infant's own potential. The protective agents in milk share several characteristics that enable them to be active in the infant<sup>6</sup>; they act at mucosal sites and are well adapted to resist the harsh environment of the gastrointestinal tract [hydrolytic enzymes, changes in gastric and pH, presence of bile salts]. Furthermore, action on microorganism is often accomplished synergistically and protection is achieved without triggering inflammatory reactions.

Non immune protection is provided by a large number of components in human milk that contrary to immune protection against specific antigens provided by milk immunoglobulins, protect in a non specific way and thus provide a broad spectrum of anti infective activity<sup>17-23</sup>. One of the proteins contained in milk-Lactoferrin has broad antimicrobial properties<sup>24</sup>.

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