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EDITORIAL

The compilation and publishing of the Journal of the Pediatric Infectious Disease Society of the Philippines has unfortunately been delayed. Thus my apologies to the authors who have submitted their original works as well as to our readers. Those who have been loyal followers of this academic journal shall not be disappointed with this issue because of the important contribution of each research article to the knowledge and practice of pediatrics.

The first article is an in vitro study comparing the antimicrobial activity of breast milk and 2 different probiotic commercial formulas. This study would make breastfeeding advocates ecstatic for it confirms the superiority of breast milk in the inhibition of the growth of 5 common bacterial pathogens-namely *K. pneumoniae, E. coli, P.aeruginosa, S. tyhpi* and *S. aureus* compared to the two commercially available milk preparations tested. Being an in vitro study, other potential benefits of probiotics such as inducer of the immune system cannot be observed.

The second article describes the clinical profile of pediatric patients with rotavirus diarrhea seen at the Philippine General Hospital. This research is not only gives us a clearer picture of the incidence of rotavirus in our country as well as the common presenting signs and symptoms, but this article makes for good reading because it gives a very concise review of rotavirus itself. It describes the history, structure, transmission and incidence of rotavirus in other parts of the world adequately giving the reader an excellent synopsis of the disease.

The third article is an experimental animal study on the effect of povidone iodine on the urinary bladder mucosa. Although a small sample size was used, it gives an accurate picture (histologic, at that) on the trauma that such a solution instilled for treatment of persistent urinary tract infections may produce. These findings show that his particular practice, which although may be effective, may potentially cause harm to the patient. Thus such a practice of instilling povidone-iodine through the indwelling catheter should probably be viewed as a procedure of last resort in such cases.

The fourth article describes the clinical profile and outcome of pediatric patients with *S. aureus* pneumonia. This particular bacteria is still a major cause of morbidity and mortality in all age groups in both community and hospital acquired infections. It is good to note that the incidence of oxacillin resistant disease is not very high and old reliable anti-Staph drugs can still be used, specially if the illness is community acquired.

The fifth article discusses the antimicrobial usage of physicians for upper respiratory tract infections in an urban setting. It has long been our suspicion that antibiotics have been abused for this indication and this is further confirmed by this study. What practitioners should note is that majority of the parents are willing to go home from the clinic without a prescription of an antibiotic as long as adequate explanation is given. Thus, physicians should not be pressured into prescribing antibiotics for fear that the patient will not be brought back for follow-up.

The next study describes the clinical profile of patients with *Burkholderia pseudomallei*, a pathogen not commonly seen in the community. This seems to be a nosocomial outbreak in a hospital wherein this bacteria may have been disturbed from the environment due to ward renovations/cleaning or was it just acquired in the community and spread to other susceptible patients? The signs and symptoms were similar to other nosocomial infections thus it was only identified by isolation of the pathogen in the blood. Fortunately in succeeding months there was a disappearance of the disease, hopefully never to be seen again in this particular hospital.

The last study elucidates in a randomized control trial some benefits of adjunctive therapy with cranberry juice in addition to antibiotics for recurrent UTI. It was shown that symptoms may be alleviated by this regimen. There was a significant decrease in duration of fever vomiting and hypogastric pain in this patient population.

The editors have also decided that to fully benefit the reader, each issue shall be given for FREE to the attendees of the PIDSP convention (of course to the first few hundred registrants). In the future, we will also be able to update the PIDSP website and articles may be downloaded on the internet also for free. Please watch out for more developments from the editors.

Cecilia C. Maramba-Untalan, MD

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THE IN VITRO ANTIMICROBIAL ACTIVITY OF PROBIOTIC CONTAINING MILK AND MATURE BREASTMILK AGAINST 5 COMMON PEDIATRIC BACTERIAL PATHOGEN ISOLATES IN MANILA DOCTORS HOSPITAL

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Abstract

The in vitro antimicrobial activity of two probiotic-containing milk formula were investigated. This was done by measuring their respective zones of inhibition using the disc plate diffusion technique against five pathogen isolated from Manila Doctors Hospital namely: *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus.*

The objectives of the study are:

1) **to determine if the two probiotic containing milk have in vitro antimicrobial activity against the five pathogens;**
2) **to compare their antimicrobial activity with that of mature breastmilk; and**
3) **to compare the antimicrobial activity of the two different probiotics containing milk.**

Results show that milk containing a combination of probiotics *Bifidobacterium lactis* and *Streptococcus thermophilus* has an in vitro antimicrobial activity for three of five pathogens tested and was comparable with mature breastmilk in their zones of inhibition against *Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus.* The milk containing a combination of probiotics *Lactobacillus salivarius* and *Bifidobacterium* has in vitro antimicrobial activity for all five tested pathogens and was comparable to mature breastmilk for *Salmonella typhi* and *Staphylococcus aureus* the two probiotic containing milk have comparable in vitro antimicrobial activity for *Salmonella typhi* and *Staphylococcus* but with significant differences ($p<0.05$) for *K. pneumoniae, E. coli* and *P. aeruginosa.*

INTRODUCTION

Bacteria are usually associated with illness. However, studies have shown that some microorganisms may actually be beneficial to human bodily function and may help fight disease. These are "friendly" bacteria and when taken as food supplement are called "probiotics"[1]. In humans, the major source of probiotics is from dairy based foods[2], like yogurt, cheese and skim milk. Probiotics have also been recently introduced in some brands of powdered milk.

Keywords: *antimicrobial activity of probiotic milk*
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This concept of friendly bacteria dates back to 1900 when a Russian physiologist Ellie Metchnikoff introduced his "intoxication theory". He attributed the health and unusual longevity of Russian peasants to the lactic acid producing bacteria in yogurt[3]. Since then, several researches have been done, usually focusing on lactobacilli. One of the most cited roles of lactobacilli is in the treatment of diarrhea, especially that caused by rotavirus[4], and relief of lactose intolerance[5]. Less established benefits of probiotic bacteria include the prevention of cancers like breast, colonic and prostate carcinoma[5], reduction of blood pressure and serum cholesterol[4], amelioration of allergic reactions[6], and fighting respiratory infections[11]. Gorbach also demonstrated that probiotics enhance macrophage activity and phagocytosis as well as humoral immune response[1].

Despite its apparent therapeutic and prophylactic attributes, probiotics are not part of a medical practitioners usual array of therapeutic agents. This skepticism is rooted in the paucity of convincing scientific validation of the efficacy of probiotics in humans.

Pediatricians have always recommended the use of breastmilk because of its known advantages against infection. Breastmilk contains lysozymes, lactoperoxidase, lactoferrin, interferon, complement components, immunoglobulins, leukocytes and anti-staphylococcal factor[7]. A study done in Philippine General Hospital – NICU comparing the activity of colostrum and broad spectrum antimicrobials against common nursery pathogens showed colostrums was comparable to Amikacin and better than Netilmycin against *Enterobacteriaciae* and was also comparable to Piperacillin – Tazobactam and Amikacin against *Klebsiella pneumoniae* and *Escherichia coli*[8]. Another study by Jayme showed that all three types of breastmilk whether colostrum, transitional or mature milk, have antimicrobial activity against eight common bacterial pathogens. These include *Escherichia coli, Haemophilus influenzae, Streptococcus pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus and Klebsiella pneumoniae*. Furthermore, the study showed that

colostrum and transitional milk do not differ in its antimicrobial properties against six of the microorganisms tested with the exception to *S. typhi* and *K. pneumoniae*. Transitional milk was also comparable to mature milk in six of the microorganisms tested except for *E. coli* and *K. pneumoniae*. When colostrums was compared with mature milk there was a significant difference in their antimicrobial effect against seven of the microorganisms except for *P. aeruginosa*[9].

The proven advantage of breastmilk is the reason why nutritional companies have been trying to make milk formula similar to breastmilk as much as possible. A recent addition to milk formula are the use of probiotics like *Bifidobacterium lactis, Streptococcus thermophilus, Bifidobacterium* and *Lactobacillus salivarius*. Some milk formula also added prebiotics in the form of Fructooligossacharides (FOS) which is the preferred food of probiotics such as *Lactobacilli* and *Bifidobacteria*.

This study was conducted to determine the in vitro antimicrobial activity of milk formula containing probiotics and compare it to mature breastmilk based on measurement of zone of inhibition.

GENERAL OBJECTIVE

To determine if milk containing probiotics (*Bifidobacterium lactis / Streptococcus thermophilus* and *Bifidobacterium / Lactobacillus salivarius*) has an in vitro antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Staphylococcus aureus* by measuring their respective zones of inhibition.

SPECIFIC OBJECTIVE

To measure the zones of inhibition of milk containing probiotics against the five common pathogens isolated at MDH using the disc plate diffusion technique.

To measure the zones of inhibition of mature breastmilk against the five common pathogens and to compare these measurements with that of milk containing probiotics. To compare the zones of inhibition of probiotics containing *B. lactis* and *S. thermophilus* versus probiotic containing *Lactobacillus lactis* and *Bifidobacterium* in the five pathogens.

METHODOLOGY

TEST ORGANISMS

The five leading bacterial isolates from blood cultures of patients admitted at Manila Doctors Hospital were identified and isolated by reviewing the blood culture and sensitivity logbook from June 2000-2001. These included *Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Staphylococcal aureus*. Reference strains of each identified organism were prepared in a broth culture done by the senior medical technologist from MDH Laboratory Bacteriology section.

CULTURE MEDIA

Mueller Hinton Agar was the medium employed using the disc plate diffusion technique for determining susceptibility of microorganisms to antibiotics. Standard techniques were used to prepare this medium.

The culture medium was prepared by mixing eight grams of commercially prepared Mueller Hinton nutrient agar per 500 ml of distilled water in an Erlenmeyer flask. The flask was then placed over a hot plate and the nutrient agar was constantly mixed with a stirring rod until the mixture became clear. The flask was then covered with a sterile cotton plug. The sterilized Mueller Hinton nutrient agar was poured on sterilized Petri dishes and were allowed to solidify. The agar plates were incubated for 24 hours at 37 ^{0}C to check for growth of contaminants.

SUBJECTS

Breastmilk samples were from ten healthy breastfeeding mothers who were consulting at the OPD-MDH. Breastfeeding routine can affect the breastmilk composition with fat content highest in the early morning for those who feed their child in the evening. Mothers who breastfeed in the evening were included in the study and samples were collected in the morning to avoid diurnal variation of breastmilk content. A consent form was issued and mothers were made to fill up a data sheet. All mothers were well nourished and had no history of infection during their course of pregnancy. The subjects were instructed to wash and scrub their hands with soap and rinsed thoroughly with water for 10 minutes. The areola and nipples were then cleaned using wet sterile cotton balls. The collection was done by stripping method. This is similar to clean catch technique in urine sample collection, wherein the first ten (10) drops of breastmilk are discarded and the next 2 ml are collected in sterile plastic containers to avoid loss of viable leukocytes which adhere to glass containers[10]. The samples were labeled from 1-10 and immediately brought to the

microbiology laboratory.

The two milk formulas containing different kinds of probiotics were prepared as instructed by the manufacturer. Sterile water and containers were used in preparation of the milk. Two (2) ml of each formula were transferred to sterile plastic containers using sterile 3 ml syringes. Each was labeled accordingly from eleven to twenty (11-20).

Autoclaved 6 mm Becton Dickinson blank discs were dipped into the prepared samples of breastmilk and milk containing probiotics, then placed into the Mueller Hinton agar medium streaked with the reference bacterial strains. The discs were then incubated for 24 hours. The antimicrobial effect was determined by the presence of zones of inhibition for all the agar plates by the one senior medical technologist who was blined to avoid bias in measurement of the disc. Blinding was done by the researcher by placing milk samples in a similar container with 2 cc of milk and were labeled from one to twenty.

The zone of inhibition was measured using a caliper with an accuracy of 0.05mm and recorded. The zone of inhibition is the clear area around the disc observed on the agar streaked by the reference bacteria and would signify the area that was not colonized by the reference bacteria.

The recorded value of zone of the inhibition was analyzed using Kruskall Waales test and the one way anova test. Comparison of the median zones of inhibition between the 3 groups was compared using the Kruskall Waalis test with a significance at α 0.05 level of significance.

RESULTS

This study included ten healthy breastfeeding mothers with age ranging from 19 to 38 years. Thirty percent were primipara and seventy percent (70%) were multigravid; sixty percent (60%) were exclusively breastfeeding their babies while forty percent (40%) were on mixed feeding. The mothers were all breastfeeding their child in the evening and with post partum age ranging from 4 to 56 weeks (mean = 24.3). Sixty percent (60%) were taking multivitamins at the time of admission. (Table 1)

Table 2 shows the comparison of the mean zone of inhibition of each type of milk when tested with the five bacterial pathogens. The zone of inhibition is a measure of the milk's activity to the reference bacteria with a larger value representing a better in vitro antimicrobial activity against the bacteria.

Table 1: Demographic profile of the mothers

Profile		Mean	Range
Age (years)		28.3	19-38
BMI: (wt)kg)/ht		21.8	20.2-25
Post-partum age (weeks)		24.3	4-56
Sex of offspring:	Female	6	60%
	Male	4	40%
Practice of breastfeeding:			
	Exclusive	6	60%
	Mixed	4	40%
Parity:	Primipara	3	30%
	Multipara	7	70%
Mode of Delivery:	NSD	7	70%
	CS	3	30%
Taking Multivitamin:	Yes	6	60%
	No	4	40%
Regular prenatal check-up:	Yes	9	90%
	No	1	10%

Table 3 compares the zone of inhibition of mature breastmilk and milk containing probiotics. The table also compares the zone of inhibition between the two types of probiotic containing milk.

Table 2: Comparison of the zone of inhibition of bacteria between the three groups

Bacteria	Mature Breastmilk (mean +/- SD)	B. lactis and S. Thermophilus (mean +/- SD)	L. salivarius and Bifidobacteria (mean +/- SD)	P value K Waalis Test
K. pneumoniae	13.2+/-1.87	0	11.0-/-1.00	<0.001(S)
E. coli	16.1+/-5.04	0	11.2+/-0.45	<0.001(S)
Salmonella typhi	14.1+/-2.08	14.6+/-0.55	13.8+/-0.45	>0.05(NS)
P. aeruginosa	17.0+/-4.76	17.0+/-1.00	13.2+/-0.55	<0.05(S)
S. aureus	15.2+/-3.16	15.2+/-0.84	15.4+/-0.55	>0.05(NS)

S = Significant NS = Not significant

Table 3: P-values for the comparison of two means by Duncan test

BACTERIA	Mature breastmilk Vs B. lactis and S. Thermophilus	Mature breastmilk Vs L. salivarius and Bifidobacterium	B. lactis & S. Thermophilus Vs L. salivarius & Bifidobacteria
K. pneumoniae	<0.001(S)	<0.001(S)	<0.001(S)
E. coli	<0.001(S)	<0.001(S)	<0.001(S)
S. typhi	>0.05(NS)	>0.05(NS)	>0.05(NS)
P. aeruginosa	>0.05(NS)	<0.05(S)	<0.05(S)
S. aureus	>0.05(NS)	>0.05(NS)	>0.05(NS)

S = Significant NS = Not significant

DISCUSSION

Breastmilk contains nutrients required newborns and infants and is a good source of non-nutritional components that promote health, growth and development, such as antimicrobial factors and digestive enzymes. The composition of breastmilk is not uniform and the concentration of its many constituents change during the lactation period between individual mothers. Factors that are known to influence the concentration of breastmilk constituents are stage of lactation, breastfeeding routine, parity, age and diet of mother[13].

Mature breastmilk is milk secreted by the mother from 15 days to 15 months post partum. Its composition can vary during the day and varies from the beginning to the end of the feeding. The fat content of breastmilk can change by as much as fivefold during the course of a feeding[12].

This study showed that mature breastmilk and milk containing probiotic *Lactobacilli* and *Bifidobacterium* has in vitro antimicrobial activity against *K. pneumoniae, E coli, S. typhi, P. aeruginosa* and *S. aureus* as evidenced by their zone of inhibition. The results further reinforced previous studies of the antimicrobial properties of breastmilk. The contents of breatmilk that confer antimicrobial properties are immunoglobulins particularly secretory IgA; lactoferrins; lysozymes; T and B lymphocytes, phagocytic cells and complement components[7]. Secretory IgA contains antibodies against *E. coli* hence, breastfed infants are less prone to enteric infection than bottle-fed infants. The amount of IgA is 3 times more in colostrums than in transitional and mature milk[11]. Human milk also contains bifidus factor which promotes growth of *Lactobacillus bifidus* which competes with invasive gram negative enteric organism[12].

One milk formula uses a combination of *Bifidobacterium lactis* and *Streptococcus thermophilus*, while another milk formula uses a combination of *Lactobacillus salivarius* and *Bifidobacterium*.

The results of the study showed that milk containing probiotic *B. lactis* and *S. thermophilus* have in vitro antimicrobial activity for *S. typhi, P. aeruginosa* and *S. aureus* only. When the zones of inhibition were compared with mature breastmilk, it showed comparable zones of inhibition with mature breastmilk.

On the other hand, milk containing probiotics *L. salivarius* and *Bifidobacterium* showed zones of inhibition for the five pathogens tested and measurements comparable to mature breastmilk for *S. typhi* and *S. aureus*.

When the two probiotic containing milk formula were compared, the zones of inhibition were significantly larger in milk formula containing *B. lactis and S. thermophilus* for organisms *K. pneumoniae* and *E. coli*. While for milk formula with *L. salivarius* and *Bifidobacterium* the zone of inhibition was significantly larger for *P. aeruginosa*.

Probiotics generally exhibit antimicrobial activities from the following postulated mechanism of action:

1) direct effects on population and activity
2) stimulate immunity
3) compete for adhesion receptors in the intestinal mucosa[13].

Bifidobacterium are anaerobic non-spore forming gram positive bacilli that are commonly found in milk and yogurt. *Bifidobacterium* have been found to exhibit inhibitory effect on many pathogenic organism both in vivo and in vitro conditions, including *Salmonella, Shigella, Clostridium, S. aureus, Candida albicans, Campylobacter jejuni and Bacillus cereus*[15].

Bifidobacterium have been shown to lower pH of the GIT by breaking down non-digestible carbohydrates to produce short chain fatty acids (SCFA). Examples of SCFA include acetate, propionate, butyrate and lactate.[14] The SCFA produced can lower the pH of the gastrointestinal tract making it less favorable for harmful bacteria. *Bifidobacterium* are also efficient competitors of pathogenic intestinal bacteria and yeast for nutrients hence controlling their populations[16].

Lactobacilli like *Bifidobacterium* are anaerobic non-sporeforming gram positive bacilli which can increase the acidity of the gut by producing lactate hence inhibiting growth of pathogenic bacteria.[17] Lactobacilli also produce hydrogen peroxide which contribute to their antimicrobial activity.

Lactobacilli also compete for adhesion receptors in the intestine. They have been shown to inhibit the activity of *Salmonella typhi* and *Listeria monocytogenes*[19]. Pathogenic organisms need to adhere to the intestinal mucosa in order to colonize the intestine. Otherwise, they are flushed from the system by the peristalsis of the gut. Probiotic organisms attach preferentially to the gut wall and prevent the pathogen from adhering to the gut. Further study by Berent et al, showed that *Lactobacillus acidophilus* is very adherent to the microvillus membrane of the human enterocytes in vitro[18], while a study by David Marek showed *Lactobacilli* inhibit adherence of *E. coli* to intestinal

mucosal cells in culture[19].

Several studies have shown that *Lactobacilli* have therapeutic properties and are able to produce antibiotic like substances as well as increase the level of immunoglobulins. A study by Perdigon and colleagues in Argentina showed that ingestion of *Lactobacilli* increases the antibody level like secretory Ig A[20]. Bifidobacterium have also been found to stimulate the production of Ig A in the small intestine and in the peyer's patches resulting in increased intestinal mucosal immunity[16,21]. Gorbach, using Lactobacilli LGG described that it has a number of immune effects demonstrated in animal models by enhancing macrophage activity, phagocytosis as well as stimulating of humoral immune response[22].

Another probiotic used is *Streptococcus thermophilus* which are facultative anaerobic, gram positive cocci that form large amounts of lactic acid by releasing lactase enzyme and consequently increasing the gut acidity[4].

A study by Chandrapati et al showed that *S. thermophilus* reduced the symptoms of lactose intolerance because of the lactase, the enzyme that aids in lactose digestion[23].

At present, continued research is being conducted to show the beneficial effects of probiotics in an attempt to decrease the incidence of diseases. But the exact mechanism by which probiotics exert their effects are still largely unknown[20].

Ideally, breastmilk is recommended for children up to 2 years old because of its already established advantage over milk formula but despite this, a study by WHO showed a declining percentage of mother who continue to breastfeed at 6 months old. Hence a search for possible supplements that would offer anti-infective properties and boost the immunity of children is still very much needed.

CONCLUSION

1. Breastmilk has in vitro antimicrobial activity against *K. pneumoniae, E. coli, S. typhi, P. aeruginosa* and *S. aureus*.
2. Milk containing probiotics *Lactobacillus salivarius* and *Bifidobacterium* has in vitro antimicrobial activity for all five pathogen tested.
3. Milk containing probiotics *Bifidobacterium lactis* and *Streptococcus thermophilus* has in vitro antimicrobial activity for three out of five pathogens tested namely *S. typhi, P. aeruginosa* and *S. aureus*.
4. Milk containing probiotic *Bifidobacterium lactis* and *Streptococcus thermophilus* is comparable to mature breastmilk in three out of five pathogens in its in vitro antimicrobial activity.
5. Milk containing probiotic *Lactobacillus salivarius* and *Bifidobacterium* is comparable to mature breastmilk in two out of five pathogens in its in vitro antimicrobial activity namely *S. typhi* and *S. aureus*.
6. Milk with *B. lactis* and *S. thermpohilus* have significantly greate zone of inhibition than the other probiotic containing milk for *P. aeruginosa*.
7. Milk with *L. salivarius* and *Bifidobacterium* have significantly greater zone of inhibition for *K. pneumoniae* and *E. coli* as compared with the other probiotic containing milk (*B lactis* and *S thermophilus*).

RECOMMENDATION

This study using in vitro method cannot fully investigate the clinical application of probiotics. It can only suggest preliminary antimicrobial activity of certain probiotics on the organisms tested. The researcher recommends further studies using in situ and in vivo methodologies. Another recommendation is to compare the antimicrobial effect of other probiotics using antibiotics.

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