Chapter

Prophylactic and Therapeutic Role of Human Breast Milk Proteins and Bioactive Peptides against Neonatal Bacterial Infections

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Abstract

Breast milk represents nature's best mechanism to provide complete nourishment and protection to the newborn. Human breast milk acts as a store house of an array of bioactive factors, which includes antimicrobial proteins and antimicrobial peptides that confer early protection while lowering the incidence of developing various infections and exhibiting immune modulation property to activate the immune cells to fight against the invading pathogens. Among the bioactive peptides, endogenous peptides present in breast milk have opened a new window of research on studying their unique mechanisms of action. This will help in incorporating these peptides in formula milk for meeting special needs where breastfeeding is not possible. The present chapter aims to give a deep insight into the various antimicrobial peptides and the newly reported endogenous peptides in human breast milk with emphasis on their levels and activity in preterm milk as data related to this is lacking and preterm newborns are highly vulnerable to acquire infections. Further, the chapter focuses on highlighting the antibacterial mechanisms adopted by the bioactive peptides for protection against the neonatal bacterial pathogens with special emphasis on the infections caused by resistant bacterial strains in hospital settings (neonatal wards) and their future implications.

Keywords: breast milk, neonatal infections, antimicrobial proteins, antimicrobial peptides, preterm, necrotizing Enterocolitis

1. Introduction

Breastfeeding provides a nursing infant with a plethora of bioactive molecules evolved to optimally develop the infants overall health. Traditionally breast milk was considered to serve only as a source of nutrition but breast milk is actually a perfect store house of an array of bioactive molecules essential for the overall development and protection of the newborn. It is considered as the best functional food for all instances, whether preterm or full term that is produced specifically by each mother to satisfy her unique infant. World Health Organization (WHO)

recommends exclusive breastfeeding until 6 months and continuation of breastfeeding until 2 years as part of mixed diet [1, 2]. Past studies clearly indicate that as compared to formula fed infants, breast fed infants present lower incidence of microbial infections, better quality and composition of their gut flora, better cognitive functions, reduced risk of allergy with a stronger immunity to fight against infections in their future as well [3–6].

These properties and benefits of breastfeeding are due to the presence of multitude of bioactive molecules in human breast milk that help to protect the newborn from pathogenic microbes while strengthening infant's immune system. Among the major bioactive molecules are the milk proteins present. Colostrum has high concentration of proteins, is low in fat and carbohydrates than mature milk indicating that its primary purpose is to provide immediately as many bioactive proteins and peptides to the newborn aimed for its protection against microbial insult [7, 8].

These bioactive proteins serve various physiological functions which include enhancement of nutrient absorption by specific binding proteins facilitating the uptake of nutrients, assistant in digestion and growth stimulation. One of the major role of bioactive proteins is their antimicrobial effect as these molecules have broad spectrum antimicrobial activity in vitro against bacteria, viruses, and fungi, as well as synergistic activity with conventional antibiotics [9–11].

Antimicrobial proteins are multifunctional defense molecules that are highly concentrated in early lactation and decrease with progressing lactation. Their composition in milk changes to serve to the growing and changing needs of infant. They present a strong defense against the pathogens thus protecting the highly vulnerable infant while its immune system is still being developed. Antimicrobial proteins (**AMPrs**) and antimicrobial peptides (**AMPs**) exhibit their effect through many different mechanism and unique ways. They are involved in (a) direct outright killing the microbes, (b) neutralizing the bacteria and viruses and making them ineffective or (c) indirectly by blocking the initial attachment/adherence of the bacteria to mucosal surfaces. Besides this, these molecules also modulate the immune system by activating immune cells against infectious agents and strengthening the innate system against life-threatening infections and those targeting the mucosal lining (gastrointestinal infections, skin infections, respiratory tract infections) [12–14]. The combination of immunomodulation and antimicrobial factors help the child to avoid the development of various childhood infections and inflammatory diseases. Another major role played by breast milk proteins is the development of infant's gut flora exerted due to the prebiotic effect [10, 15]. Therefore, breast milk is a unique and complex reservoir of multitude of proteins and peptides all of which work in a synergistic manner to maximize the benefits in favor of the growing infant.

Another area of interest pertains to endogenous peptides present in human milk and their unique role that still warrants further research. These peptides are derived from proteins by specific proteases, many possess antibacterial action [16, 17]. Studying such endogenous peptides especially their role in preterm milk is of paramount importance in developing functional foods to cater to the special need of low birth weight infants (LBW) as well as preterm infants that stand at a higher chance of developing infections than term infants.

The role of milk derived antimicrobial proteins and peptides against neonatal pathogens and their clinical utility is definitely a complex topic and needs more detailed investigation. The present chapter focuses on various bioactive proteins and peptides involved, their mechanisms, spectrum of activity against range of bacterial infections with special emphasis on preterm infants and role of these molecules against resistant infections that are on rise in neonatal wards. Finally, future implications pertaining to use of newer technologies to exploit various

human milk derived proteins and novel peptides as therapeutic and preventive intervention strategy has been highlighted.

2. The human milk proteome

Human breast milk is an ideal nutrition to the infants consisting of 87% water, 1% protein, 4% lipid and 7% carbohydrate along with various minerals and vitamins [15]. These bio-components of breast milk have been shown to provide bioactivities that are important for infant growth and development. The largest variety of bioactivities, however, is provided by proteins in breast milk [18].

Breast milk contains a wide array of proteins which are present in the form of enzymes/proteases, glycoproteins, and endogenous peptides. They provide unique biological activities, ranging from antimicrobial effects to immunostimulatory functions, facilitating the digestion and uptake of other nutrients in the milk such as iron, calcium and vitamin B besides providing sufficient amounts of essential amino acids to breast-fed infants [8]. The concentration and composition of human milk proteome changes continuously in their composition and concentration throughout gestational age and lactation stage. Over the last several years, an extensive analysis of the protein composition of term and preterm breast milk has been done [19–21]. Results indicate both quantitative as well as qualitative modifications through lactation in both term and preterm breast milk. Breast milk obtained from mothers who deliver preterm showed significantly higher protein content than that of mothers who deliver at term [22, 23]. The mean total protein content in preterm milk has been reported to range from 3.0 to 1.9 g/dl, and in term milk to vary from 2.2 to 1.1 g/dl over the first 4 weeks postpartum [19, 20, 24–26]. As lactation stage proceeds from colostrum to transitional milk, a gradual and physiological decrease in protein quantity occurs in both kinds of milk [26–28]. A decrease of protein levels by 30% was reported in preterm milk and by 50% in term milk from the first to eighth weeks of lactation [20, 29]. The concentration of proteins remains relatively constant thereafter in mature (term) milk. However, according to Lucas and Hudson [30], the volume of milk produced is an important determinant of protein content. They negatively correlated the postnatal age of the donor with milk protein concentration. Also, for feeding preterm infants, the lower level of total protein and specific amino acids from donor (typically, term, late lactation) milk alone is limiting, and requires additional supplementation. Many other factors also influence the protein content of human term milk. Bachour et al. [31] showed that smoking and mother's basal metabolic index (BMI) and lactation stage significantly decreased the protein content (12%) of the term milk. Overweight mothers also showed a lower milk protein concentrations [32]. The treatment of expressed milk also induces changes in the valuable nutrients contained in human milk. Ramirez-Santana et al. [33] evaluated the effect of cooling storage at 4°C and freezing storage at −20°C and −80°C on bioactive factors in human colostrum. The results interpreted that colostrum can be stored at 4°C for up to 48 h or at −20°C or −80°C for at least 6 months without losing its immunological properties provided by bioactive proteins. Similar study reported that lactating mother can pump the milk and refrigerate it for later consumption without compromising on the antibacterial potency of their milk against for up to 24 h [34].

Bjorksten et al. [35] and Evans et al. [36] reported no significant changes in human term milk proteins after freezing for 3 months. Another study suggested that frozen storage resulted in a lower reduction in various bioactive proteins as compare to pasteurization in term milk [37] Similarly, Chang et al. [38] revealed a non-significant decrease in most of the bioactive proteins in frozen as well as in

low-grade heat treated (below 60°C) term milk. They proposed frozen breast milk as an alternative choice if fresh breast milk is unavailable. The Academy of Breastfeeding Medicine has a protocol for home storage of human milk that can be used to guide mothers in these activities to optimize the integrity of expressed and stored milk.

3. Breast milk and antimicrobial proteins

Human milk possesses inherent antimicrobial proteins that have been attributed to the defense against number of pathogens preventing their proliferation and invasion. The major antimicrobial proteins (AMPr) present in human milk act as first line of innate defense and are discussed below:

3.1 Lactoferrin

Human lactoferrin (HmLf) a multifunctional whey class of globular glycoprotein, found in abundance in human milk. The concentration is highest in colostrum at 5.5 g/L and decreases between 1.5 and 3.0 g/L in mature milk depending on the stage of lactation [39]. The decline in lactoferrin concentration is slower in preterm mother's milk than in full-term mother's milk [40]. Lactoferrin is an antiviral, antibacterial and anti-inflammatory protein. HmLF exert antimicrobial activity against Gram-positive [41–44] bacteria by either iron-depletion and/or disruption of bacterial membrane [45]. Lactoferrin also possesses anti-inflammatory properties and seems to be involved in phagocytic killing and immune responses [46]. The presence of human lactoferrin in breast fed infants indicate that it survive proteolytic digestion. Higher concentration found in the feces of premature infants suggests that these proteins play an active role in the infant gut [47].

3.2 Secretory immunoglobulin A (sIgA)

sIgA is the major immunoglobulin belonging to whey group of proteins present in human milk. The average concentration ranges from 2.0 g/L in colostrum to approximately 0.5 g/L in mature milk [48]. The secretory component works as a defense mechanism for the antibody molecules, protecting them from gastric acid and digestive enzymes, hence, resist digestion [49]. As a result, sIgA molecules remain active throughout the infants' gastrointestinal tract and protect against bacterial infections especially diarrheal diseases which are a major cause of morbidity and mortality in children in developing countries [50]. sIgA bind to pathogenic bacteria, toxins and other antigenic materials, such as lipopolysaccharide (LPS), thus, preventing their adherence and penetration into the intestinal epithelium without triggering inflammatory reactions that could be harmful to the newborn. This mechanism is called immune exclusion.

Apart from direct binding, sIgA can agglutinate bacteria non-specifically through oligosaccharide α-side chains of the immunoglobulin molecule. These oligosaccharides bind to fimbrial lectins, e.g., of *E. coli* and other bacteria [51]. Purified sIgA from human milk has been shown to protect the breast-fed infant by inhibiting the adhesion of enteropathogenic *Escherichia coli* (EPEC) to HEp- 2 cells in cell culture [52]. Another study demonstrated the ability of human milk sIgA to block the adhesion of *Staphylococcus aureus* strain in tissue culture.

3.3 Lysozyme

This protein constitutes a major fraction of the whey protein of breast milk. It is present in higher amounts in colostrum (0.36 g/L) and its concentration is slightly reduced in mature milk to 0.30 g/L [53]. Lysozyme is a protein that can exerts its antibacterial effect against gram positive bacteria independently by cleaving the 1–4 linkage between N-acetyl glucosamine and N-acetylmuramic acid in their cell wall. It may also act in concert with lactoferrin to kill both gram-positive and gram-negative bacteria and perhaps also against viruses. Electron microscopic studies showed that lactoferrin first binds to LPS in outer membrane of the bacteria creating holes through which lysozyme can penetrate and degrade the inner peptidoglycan matrix [8]. It is found intact in the stool of infant showing that it may exert its activity in the gut of breast fed infant.

3.4 κ-Casein (κ-CN)

k-casein, a small subunit of casein present in breast milk, is a highly glycosylated (90%) oligosaccharide. Concentration of kappa casein in colostrum is 25 and 1 g/L in mature milk [54]. The antimicrobial properties of the κ-casein portion of human milk fluids were first reported by Aniansson et al. [55]. κ-casein was showed to inhibit the adherence of *Streptococcus pneumoniae* to human respiratory tract epithelial cells in vitro. κ-casein prevents the attachment of bacteria to the mucosal lining as their oligosaccharides act as a receptor analogue having structures similar to exposed glycans on mucosal surface [56]. Others have demonstrated an inhibitory effect of κ-CN on the adhesion of *Helicobacter pylori* to human gastric mucous cells [57]. These studies clearly established that the actual anti-infective agent responsible for preventing infection in their respective cell types was the carbohydrate portion of the glycoprotein. Later, the fucose carbohydrate moiety was specifically identified as the primary factor responsible for inhibiting adhesion. Studies have also indicated that κ-CN in milk works together in a synergistic approach with lactoferrin, and sIgA work to protect the new born against microbial attack.

3.5 Osteopontin (OPN)

It is a multifunctional glycosylated and heavily phosphorylated acidic protein initially discovered in bones. With high variability among mothers and stages of lactation, the average concentration of osteopontin in breast milk is approximately (138 ± 79 mg/L, mean $\pm$ SD) [58]. Present in low concentration in colostrum but after 3 days of lactation, high levels are established. The levels decrease with advancing lactation, but about half maximal levels are maintained beyond 1 year of lactation [59]. In vitro experiments have indicated that human and bovine milk OPN are in part resistant to proteolysis in the infant intestinal tract, which makes OPN a potentially bioactive component of human milk [60]. It also plays an important role in immune activation and immune regulation by acting as chemotactic agent and stimulates both pro- and anti-inflammatory processes. It enhances B lymphocyte immunoglobulin production and proliferation and also influences cell-mediated immunity by inducing Th1 cells [61]. Furthermore, it also has been shown to form complexes with lactoferrin and act as carrier for other immunomodulator protein to enhance their competencies [62].

3.6 Haptocorrin

Haptocorrin is a heavily glycosylated protein present at a concentration of 5 mg/ml in colostrum and 3 mg/ml matured milk. Haptocorrin is a vitamin B12-binding protein, stable against proteolytic digestive enzymes [46]. As the infants lack intrinsic factor required for absorption of vitamin B12, hence, haptocorrin may facilitate vitamin B12 absorption [63]. Even at low concentration, haptocorrin has been shown to possess antibacterial activity against pathogenic strains of *E. coli* and thus may serve as a defense protein against *E. coli* related infection [64]. However, more studies delineating the effect of the bacterial strain and it's mode of action is required as its antimicrobial role has been shown to be limited to very few pathogens.

3.7 Lactoperoxidase

A member of whey protein, lactoperoxidase is secreted by mammary glands and is persistently present during lactation with concentration of 1–1.5 units/ml [65]. Lactoperoxidase is a glycoprotein and it is resistant to proteolysis [66], thus playing a role in infant host defense. It forms lactoperoxidase system with thiocyanate (SCN^-) present naturally in human milk, and H_2O_2, which is generated by bacteria [67]. In the presence of H_2O_2, lactoperoxidase oxidases SCN^- to hypothiocyanite ($OSCN^-$), which has antimicrobial activity against both Gram positive and Gram negative bacteria [68]. These chemical reactions also cause leakage of potassium ions, amino acids, and peptides across the damaged cytoplasmic membrane. Lactoperoxidase together with sIg and lysozyme help to eradicate microorganisms from the small intestine without inflammation, contributing to the development of a healthy microbiome [69].

3.8 α-Lactalbumin

The most well characterized and primary whey protein in human milk is α-lactalbumin [70, 71], which accounts for 20–25% of total breast milk proteins. During its digestion, peptides appear to be transiently formed that have antibacterial and immunostimulatory properties, thereby possibly help in providing protection against infection. Various hydrolytic products of α-lactalbumin have shown antimicrobial activity against *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus*, *Streptococcus*, and *C. albicans* [72].

3.9 Mucins

Mucins are high molecular mass glycoproteins (200–2000 kDa) heavily glyosylated proteins with variable number of tandem repeats, i.e., mucin domains [73]. Mucins are found within the milk fat globule membranes (MFGM), and typically make up less than 1% of total protein. At least 16 mucins have been identified in humans. Mucin-1 and Mucin-4 have been identified as the major human milk mucins [73]. Mucins provide protection against gastrointestinal and respiratory tracts infections by decreasing the adhesion of pathogens to the cell surface. Mucin-1 specifically has been reported to inhibit the invasion of *Salmonella typhimurium*, in a model of fetal intestinal cells, at concentrations that are similar to that of human milk [73]. Sialic acid moiety of mucin-1 interacts with pathogen thereby inhibiting the ability of the pathogen to bind to its infant host cell surface glycan receptor.

3.10 Lactadherin (milk fat globular membrane protein; MFGMP)

Lactadherin is a 46 kDa mucin associated sialyated glycoprotein found in milk fat globule membrane. In a study reported by Newburg et al. [74], 200 infants in Mexico City from birth to 2 years of age were closely monitored for rotavirus infection symptoms. Milk samples were obtained from the respective mothers weekly until 4 weeks post-partum. The milk samples were taken immediately before an infant's episode of rotavirus infection and levels of lactadherin, butyrophilin, mucin, and secretory IgA were determined. Results indicated that concentration of lactadherin in the milk samples fed to infants belonging to asymptomatic group was 48.4 (range 5.6–180) μg/mL while in the symptomatic group, it was lower, i.e., 29.2 (6.2–103.4) μg/mL. No such association between symptom status and concentrations of butyrophilin, mucin, or secretory IgA was found. These findings indicated that Lactadherin concentrations showed a significant association with symptoms in rotavirus-infected breastfed infants and is representative of a class of non-antibody glycoconjugates in human milk having protective effect against symptomatic rotavirus infection.

4. Breast milk: reservoir of antimicrobial peptides (AMPs)

Human milk derived bioactive peptides are low-density molecules (5–90 amino acids) exhibiting their bioactivity features when separated from the parental proteins. These human milk peptides not only act as sources of amino acids, but they are also involved in immune-modulation, opioid-like activity, antioxidant, antimicrobial, and antiviral action, and probiotic action. These peptides when released may express activity different from that of the parent protein. This may account for their "encrypted" role other than parent protein after digestion [75]. Nielsen et al. [76] identified a total of 5264 unique peptides by mass spectrometry deriving from human and bovine milk proteins. Of these 1722 and 3399 originated from bovine and human milk proteins, respectively. β-casein accounted for 71.2% of the total human peptide ion intensity, with αs1-casein and κ-casein combining for an additional 11.7%.

Anti-microbial peptides (AMPs) exhibit activity against an array of neonatal pathogens both Gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*, Group B Streptococcus, i.e., GBS) and Gram negative bacteria (*E. coli*, *Klebsiella pneumoniae*), mycobacteria, fungi and even the viruses [14, 77–79]. AMPs are expressed either constitutively or their expression can be inducible in response to certain pro-inflammatory stimuli. AMPs are cationic peptides that destroy bacteria in a unique way that is less prone to resistance. They initially target the bacteria via electrostatic contact at the anionic bacterial surface, i.e., AMPs interact with the highly negatively charged surface of the membrane consisting of lipopolysaccharide. This is then followed by self-promoted uptake while the AMPs insert and translocate to the outer bilayer to bind the anionic inner membrane [80]. This leads to rapid killing and lysis due to serious reduction of membrane integrity at high concentrations with some AMPs, also having intracellular targets such as DNA [81]. The concept of extracellular entrapment of bacteria by AMPs at epithelial surfaces and within the bloodstream is also one mechanism.

Although many AMPs of bovine origin have been studies in detail, but we are focused on detailing the antimicrobial peptides of human origin and their mode of action. Studying the activity and properties of human origin antimicrobial peptides will open a new window of alternate therapy targeting the treatment of infections resilient to the action of antibiotics especially in neonatal scenario. Also, such potent

peptides can be further isolated, cloned and purified to be incorporated into infant formula feed acting as a ready therapy given through food to the highly vulnerable preterm infants. The major antimicrobial peptides present in human milk and their mechanism of action (**Figure 1**) reported till date are as follows.

4.1 Defensins

Defensins (2–6 K Da) are small cysteine rich cationic proteins that participate as host defense peptides against bacteria, fungi and enveloped/non enveloped viruses. Defensins can be categorized broadly as α-defensins, β-defesins and Θ-defensins. α-defensins are produced in neutrophils and NK cells mostly in a constitutive manner whereas β-defensins are secreted by epithelial cells of various kinds which also include mammary gland. Armogida et al. [82] identified expression of hbd-2 gene in 15% of mammary epithelia cells. Baricelli et al. [83] quantified human β-defensins-2 (HBD-2) levels in colostrum and mature milk from 100 donors. The colostrum showed concentration ranging from 2.5 to 16.3 μg/ml whereas mature milk showed a low concentration of an average of 0.97 μg/ml.

They also reported that HBD-2 had potent activity against three opportunistic pathogens *Salmonella*, *E. coli* and *Pseudomonas aeruginosa*. It also showed activity of 4 *μg/ml against multi-drug resistant Acinetobacter baumanii*. These molecules have been shown to form nets to trap bacteria and combat invasion into deeper tissue [84]. These findings highlight the protective role of this peptide against serious gastrointestinal infections in neonatal population.

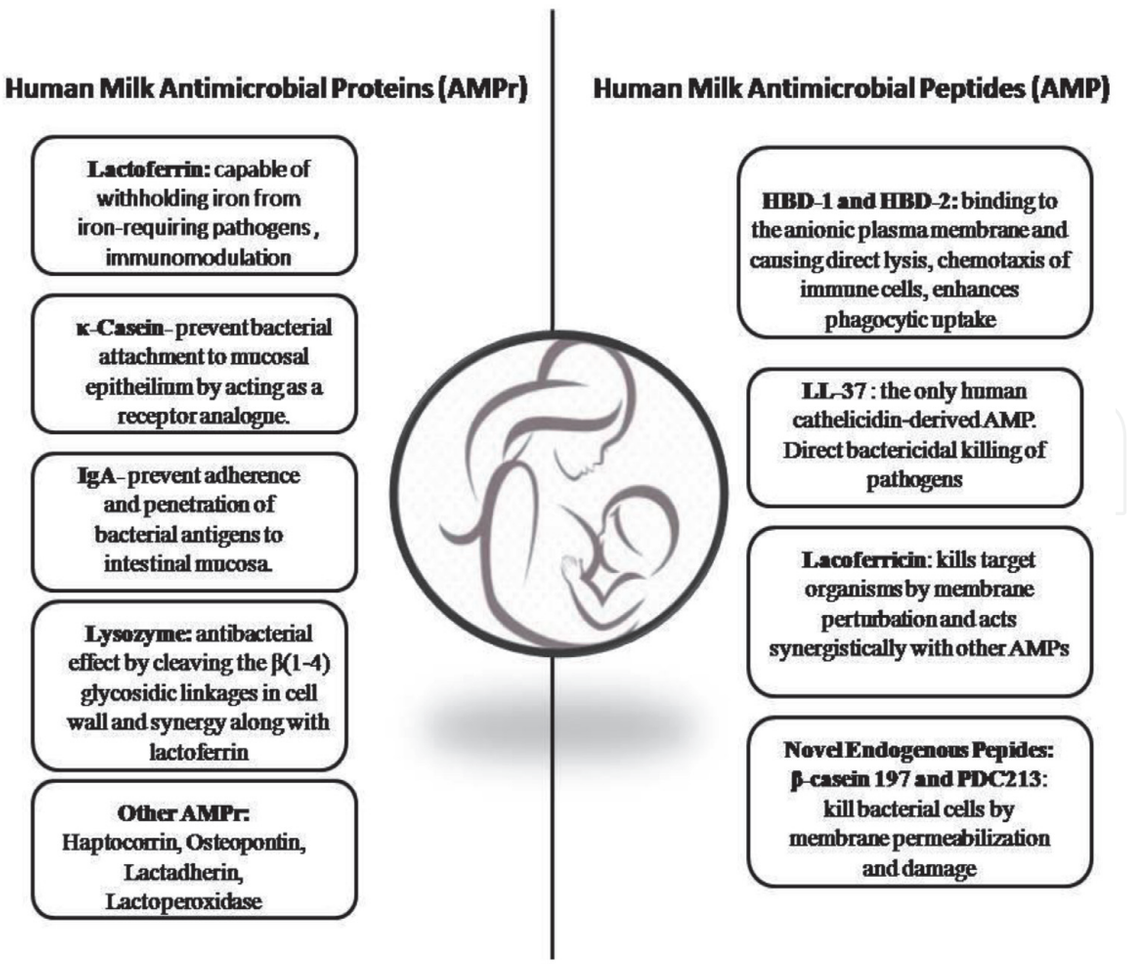

Figure 1.
Summary of different antimicrobial proteins and peptides in human Milk and their action.

Jia et al. [85] studied the levels of human β-defensins-1 (HBD-1) in human breast milk by western immunoblotting and reverse HPLC. They detected that level of HBD-1 was 1–10 μg/ml in mature milk and exhibited antimicrobial activity against *Escherichia coli*. In other study, breast tissue sampled from lactating mothers also showed hBD-1 mRNA expression in mammary gland epithelial cells and in active milk [86]. HBD1 also showed killing activity against *E. coli* at a conc. of 5 μg/ml and against *Salmonella enteritidis* through formation of entrapping nets in a redox dependent mode of action [87]. HBD-1 may act synergistically with other peptides present in breast milk. Also, HBD-1 acts as a chemotactic agent to recruit dendritic cells, T-cells to mucosal surfaces (respiratory, gastrointestinal, and nasopharynx) thus acting as a link between innate and adaptive immunity for the neonate [88].

4.2 Cathelicidins

Cathelicidins are multifunctional bactericidal peptides characterized by a highly conserved N-terminal domain of about 100 amino acid residues. This 14 kDa cathelin-like domain is flanked by a signal peptide domain (approximately 30 residues long) on its N-terminus, and by an antimicrobial peptide region on its C-terminus. The single 16 kDa human cathelicidin is denoted as hCAP18 and it becomes active only upon proteolytic cleavage into cathelin domain and cathelicidin-derived AMP yielding LL-37 [89]. This LL-37 is the only human cathelicidin-derived AMP secreted by mammary gland and present in human milk [90].

Human cathelicidin hCAP18/LL-37 mRNA expression was confirmed in human milk cells showing an increase in expression levels at 30 and 60 days after parturition. Further, western blot analysis showed that LL-37 was secreted and present in the mature peptide form in human milk and is present in expressed breast milk (EBM) of mothers of both term and preterm infants [91].

LL-37 exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria., The ability of cathelicidins and defensins to directly confer protection against bacterial colonization of epithelial surfaces has been shown in gut, lung, and skin [92, 93]. Chen et al. [94] also showed synergistic effect of antibacterial agents' human beta-defensins, cathelicidin LL-37 and lysozyme against *Staphylococcus aureus* and *Escherichia coli*. Scheid et al. [95] examined antimicrobial activity of LL-37 (15 μg/ml) in hirudin-anticoagulated preterm and term human cord blood against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Candida albicans* by CFU assay. LL-37 enhanced the antibacterial/antifungal activity against all three pathogens in term blood and against *S. epidermidis* in preterm blood.

4.3 Lactoferricin

Although Lactoferrin is not readily digested and is found intact in stool sample of infants, it is digested partially to give a peptide called "Lactoferricin". This peptide is able to inhibit adherence of *Escherichia coli* to intestinal cells [96]. Lactoferricin is also active against clinical isolates of enterohemorrhagic *E. coli* 0157H:7 at concentrations significantly less than either the lactoferrin hydrolysate or lactoferrin, itself [97]. The mechanism involved in killing the target bacteria is through membrane perturbation and this peptide also acts synergistically with other proteins and antimicrobial agents [98]. In addition to Lactoferricin, the role of Lf (1–11) has also emerged in the recent past [99] as Lf(1–11) was demonstrated to be active against gram-positive bacteria (*Staphylococcus* spp. and *Streptococcus mitis*) as well as gram-negative bacteria (*Acinetobacter baumannii*, *Pseudomonas* spp., *Klebsiella* spp., and *E. coli*) [98, 100].

4.4 Novel endogenous antimicrobial peptides

- **β-casein 197:** Fu et al. [101] used tandem mass spectrometry (MS/MS) to identify the peptides in both term and preterm human milk, and identified a peptide derived from β-casein: a sequence (197–213) from human β-casein. It is a 17 amino acid (197–213) peptide fraction of β-casein that is a newly found endogenous peptide hydrolyzed human from β-casein. It exhibits potent bactericidal property against *E. coli*, *S. aureus*, *Yersinia* spp. but no activity was seen against *Bacillus subtilis* and *Klebsiella pneumoniae* by disk diffusion assay. Electron microscopy images of treated cells revealed leaky cytoplasm and bleb like structures indicating the β-casein 197 peptide killed cells by means of membrane permeabilization instead of DNA binding.

- **PDC213:** Sun et al. [102] reported another novel endogenous peptide from human milk called PDC213. PDC213 was derived from β-casein (213–266 amino acid residue). This endogenous peptide identified by the group exhibited potent antimicrobial activity against *S. aureus* and *Yersinia enterocolitica* using in vitro assays. Furthermore, the group also found that PDC213 can effectively permeabilize the bacterial membrane to cause direct damage to the bacteria.

5. Neonatal infections: a brief overview

The first 28 days of life, i.e., the neonatal period is the most crucial and the most vulnerable time for survival of newborn. It is reported that in 2018, 2.5 million children died in the first month of their life which means 7000 neonates die each day with one-third dying on the day of birth [103]. An estimated 16 million neonatal deaths occur annually due to infection representing 40% of all neonatal deaths which is a very high percentage. Among the infections, diarrhea, pneumonia, neonatal sepsis and malaria are the leading causes [104]. Despite recent advances in neonatal intensive care and current treatment interventions, the global burden of neonatal deaths due to infections is high and represents a challenge especially in developing and poorer countries. The leading neonatal infections that account for the greatest morbidity and mortality among neonatal population have been highlighted below and in **Table 1**.

5.1 Neonatal sepsis

Neonatal sepsis remains serious complication especially in preterm and VLBW infants. Globally the greatest burden of neonatal sepsis falls in low resource developing countries [105]. It is divided as early onset sepsis (EOS) that occurs in first week of life and late onset sepsis (LOS) occurring after 1 week acquired after birth. Group B streptococcus (GBS) remains the dominant cause of EOS with 20–33% mortality in premature infants [106, 107]. *E. coli* is the second most common cause isolated from such cases especially in VLBW infants [108, 109]. LOS is largely caused by organisms acquired in postnatal period in infants exposed to invasive procedures, tubings, devices etc. Coagulase negative Staphylococci (CoNS) has emerged as one of the most commonly isolated pathogen in VLBW infants (22–65%) of LOS infection [110, 111]. *S. aureus* is associated with 4–8% of such cases of LOS. The increased incidence of MRSA isolates from neonatal sepsis is a matter of concern as 25% of infants infected with MRSA die [112].

Neonatal infections	Etiological agents (Bacterial) involved
Early onset sepsis (EOS)	Dominant: Group B Stretococcus (GBS), *E. coli* Less common: *Str. pyogenes, Str. pneumoniae, Haemophilus influenzae*
Late onset sepsis (LOS)	Commonly isolated: Coagulase negative Staphylococci (CoNS), *S. aureus* Others: *Acinetobacter baumannii, Str. pneumoniae*
Early onset pneumonia (EOP)	*Str. pneumoniae* (25% of cases), Group B Streptococcus (GBS), *E. coli*, *Klebsiella* spp., *S. aureus*
Late onset pneumonia (LOP)	*S. aureus* (dominant cause), *Str. pyogenes, Str. pneumoniae, E. coli* (less common)
VAP	Methicillin sensitive *S. aureus* (MSSA) and Methicillin resistant *S. aureus* (MRSA), CoNS, *Streptococcus pneumoniae*
Necrotizing Enterocolitis (NEC)	Members of Enterobactericea (*E. coli*, Uropathgenic *E. coli* (UPEC), *Klebsiella pneumoniae*) *Pseudomonas aeruginosa*, Coagulase negative Staphlycocci (CoNS), MRSA, *Clostridium difficle*
Infantile diarrhea	ETEC, EPEC, *Shigella flexneri*, *Salmonella* spp.
Neonatal skin infections	*S. aureus, S. epidermidis,* CoNS*, Strep. pyogenes, Strep. pneumoniae*
Other infections (Otitis media, sinusitis, conjunctivitis, urinary tract infections)	*S. aureus, S. epidermidis, Str. pyogenes Str. pneumoniae,* Group B Streptococci (GBS) *Pseudmonas aeruginosa*, *E. coli*, *Klebsiella* spp.

Table 1.
Commonly encountered neonatal infections in preterm and term neonates along with their etiology.

Among Gram negative organisms, *E. coli* (20–31% of LOS cases), *Klebsiella pneumoniae*, *Pseudomonas* spp. are also isolated with worse outcomes and high mortality seen.

5.2 Necrotizing Enterocolitis (NEC)

NEC is a common gastrointestinal emergency among preterm and very low birth weight (VLBW) infants but rare in full term infants. Its exact pathology and mechanism still has to be clearly understood. Despite advanced care of preterm infants in Neonatal Intensive Care Units (NICU), NEC remains the leading cause of morbidity and mortality in this population. It is estimated that 1–5% of all NICU admissions and 5–10% of all VLBW (≤1500 g) are affected or at risk of developing NEC with about 30% of them unable to survive their first week itself [113].

In 80% cases of NEC with intestinal perforation, members of Enterobactericeae especially *E. coli*, *Klebsiella pneumoniae* were present in peritoneal fluid in 75% of cases, Coagulase negative Staphylococci (CoNS) in 14% of cases and anaerobes in 6%. Even cases of MRSA associated with few cases of NEC have emerged [114].

5.3 Neonatal pneumonia

It is estimated that pneumonia contributes to more than 1.2 million neonatal deaths and an unknown number of still births each year worldwide. Early onset pneumonia is acquired during labor/delivery and late onset pneumonia is acquired during postnatal period from colonization of endotracheal tubing, ventilator tubing,

intravenous lines, from clinical staff and hospital staff after 48 hours or more of invasive mechanical ventilation [115]. The causative agents involved have been delineated in **Table 1**. *Streptococcus pneumoniae* and Group B streptococcus (GBS) are commonly encountered in early onset cases. Ventilator associated pneumonia (VAP) is on common rise as it has emerged as the second most reason for antibiotic intervention in NICU [116, 117] with *S. aureus* being the dominate cause due to its biofilm forming ability. Rising rates of resistance to common antibiotic means more fatality rates from neonatal pneumonia especially in poorer nations emphasizing on need to exploit alternative intervention strategies.

5.4 Infantile diarrhea

Diarrhea has been described as the leading cause of deaths due to infection in the neonatal period. It accounts for more than 10.5% of all deaths and despite efforts focused to reduce the related mortality, it still represents the main preventable cause of deaths in newborns and young children [118, 119]. The underlying cause may be due to bacterial, viral and parasitic origin. Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), *Shigella flexneri*, *Salmonella* spp. are all involved in different cases of bacterial diarrhea outbreaks more concentrated in slum areas.

In addition to this, other neonatal infections commonly encountered include conjunctivitis, skin infections (Bullous impetigo, skin abscess, scalded skin syndrome), Otitis media, urinary tract infections, etc.

6. Protective role of breast milk against neonatal infections

Early and exclusive breastfeeding is one of the most important intervention strategies to reduce the risk of developing serious bacterial infections and related infant mortality [119, 120]. The risk of non-viral diarrhea is higher for non-breast-fed infants in the first 4–6 months of their life. A recent meta-analysis suggested that infants who were breast-fed for >4 months showed a three times reduced frequency of developing severe respiratory tract infection as compared with infants who were not breast-fed [121]. In other studies, breast milk in protection against otitis media and urinary tract infections was reported [122, 123]. There is also an increased risk of mortality in children due to suboptimal or insufficient breastfeeding, which is responsible for 11.6% of total deaths of children under 5 years old [124]. Mother milk is also the best option for preterm and very low birth infants as studies indicate the over expression and increased level of range of functional peptides in preterm milk that may act as ready source of protection for the vulnerable infant. Preterm infants due to their low intake of breast milk volumes and associated proteins and peptides may be actually at higher risk of developing serious life threatening infections like NEC, late onset sepsis, pneumonia, etc. [75]. **Table 2** summarizes the finding of few of the important studies elucidating the protective role of breast milk against common neonatal infections.

The antimicrobial proteins and range of antimicrobial peptides present in breast milk act synergistically adopting multiple mechanisms to inhibit disease progression by the invading pathogen. Human milk proteins and their derived peptides have been discussed in Sections 3 and 4. Antimicrobial peptides released from any source including breast milk circulate in infants' blood stream and provide an ongoing source of low-level of non-specific immune defense against potential invasive pathogens. LL-37 along with bactericidal permeability increasing protein (BPI) has been shown to be present in higher levels in infant's blood suffering from acute blood stream infections. Also, LL-37 exhibits significant inhibitory activity against

Target infection	Reference	Objective and study design	Result	Final outcome
Neonatal sepsis	[125]	**Objective:** To investigate the protective efficacy of breastfeeding against neonatal sepsis high risk population in Lahore, Pakistan. **Study Design:** A case–control study where 42 cases of neonatal sepsis from hospital and 270 age matched controls acted as participants Exclusive breast feeding was as most babies were partially breast fed and a few given formula feed or animal milk.	In the partially breast fed group there were 19 cases and 253 controls while in the group given no breast milk there were 23 cases and 17 controls. Therefore, the incidence of breast feeding was less among the cases than the controls, with an odds ratio of 18.	Even partial breast feeding protects against neonatal sepsis in premature infants.
Neonatal pneumonia	[126]	**Objective**: To assess whether breast feeding protects young children against pneumonia and whether this protection varies with age. **Study Design:** A nested case control study in Brazil where 152 infants aged 28–364 days who had been admitted to hospital for pneumonia participated in the study.	Results indicated that the babies who were not being breast fed were 17 times more likely to be admitted with pneumonia as compared to breast fed infants and this risk was higher in the initial 3 months of infancy.	Breast feeding protects young children against pneumonia, especially in the first months of life.
Acute respiratory illness (ARI), pneumonia and diarrhea	[127]	**Objective:** To assess the potential role of exclusive breastfeeding in reducing the incidence of deaths due to acute respiratory infections (ARI) and diarrhea **Study Design:** A prospective observational study was conducted on a birth cohort of 1677 infants who were born in slum areas of Dhaka, Bangladesh and followed from birth to 12 months of age. On basis of verbal autopsy and a structured questionnaire, the mortality attributable to ARI and diarrhea was measured.	The overall risk of infant deaths from all causes was 2.23 fold higher in infants with no or poor breast feeding as compared with infants with exclusive breastfeeding while the risk of deaths due to ARI and diarrhea was still higher, i.e., 2.40- and 3.94-fold higher in no or partially breast fed babies.	Exclusive breastfeeding in the first few months of life significantly decreases onset of ARI and Diarrhea.
Lower respiratory tract illness	[128]	**Objective:** To examine breastfeeding and the risk of hospitalization for lower respiratory tract disease in healthy full-term infants with access to modern medical care. **Study:** It was a meta-analysis of 33 studies.	Result from this meta-analysis indicated that among the infants with severe respiratory tract illnesses resulting in hospitalizations, more than tripling of them were those who were not breastfed for the initial 4 months of their infancy.	Breast feeding decreases risk of lower respiratory illnesses.
NEC	[129]	**Objective**: To determine the association between human milk (HM) intake and risk of necrotizing enterocolitis (NEC) or death among infants (401–1000 g birth weight).	Results indicated 13.6% infants died and developed NEC after 14 days of their birth. However, after the initial 14 days, the	Dose dependent effect was evident between intake of human milk and reduction in risk of developing NEC and

Target infection	Reference	Objective and study design	Result	Final outcome
		Study Design: Analysis of 1272 infants in the National Institute of Child Health and Human Development Neonatal Network Glutamine Trial was performed to determine if increasing HM intake was associated with decreased risk of NEC or death.	incidence of NEC decreased by a factor of 0.83 with every 10% increase in the total intake of Human milk. Therefore, a strong dose association was seen with a decreased risk of NEC or death among infants who received 100% human milk as a proportion to total enteral intake.	related death after the first 2 weeks of life among VLBW infants.
Ventilator-associated pneumonia (VAP)	[130]	**Objective**: To explore the use of mothers' own milk (colostrums, transitional milk, and mature milk) as oral care in the ventilator-associated pneumonia (VAP)-prevention bundle of mechanically ventilated preterm infants weighing 1500 g or less. **Study Design:** Retrospective descriptive involving mechanically ventilated preterm infants weighing 1500 g or less admitted to a regional level III NICU in the Gulf South. To these, oral care with mothers' own milk was implemented as part of the VAP-prevention bundle and the outcomes that were assessed included rate of positive tracheal aspirates, positive blood cultures, the number of ventilator days, and length of stay.	Rates of positive tracheal aspirates and positive blood cultures showed reduced values in infants receiving oral care with mothers' own milk.	Use of mother's milk as part of oral care as VAP-prevention bundle is a feasible safe and effective practice and warrants further research.
Neonatal conjunctivitis	[131]	**Objective**: To investigate the effect of human breast milk (colostrums) in preventing neonatal conjunctivitis. **Study Design:** Randomized clinical trial where the intervention group with culture-negative eye swab received two drops of colostrum in each eye, antibiotic group received erythromycin ointment (0.5%), while control group received no treatment. All neonates were followed for the occurrence of clinical conjunctivitis for 28 days.	Results indicated that application of colostrum significantly decreases the onset of neonatal conjunctivitis in the test group as compared to control group ($p = 0.036$).	A positive effect with application of Human colostrums was found and thus can act as a favorable option in place of antibiotics against neonatal conjunctivitis.
NEC	[132]	**Objective**: To study the effects of feeding exclusively human milk (EHM) diet to premature infants on reducing the incidence of necrotizing enterocolitis (NEC) associated	In the control cohort, NEC onset after day 7 of life occurred in 15 of 443 infants (3.4%), significantly more than in the EHM cohort	Changing to an EHM milk diet through 33 weeks PMA reduced the incidence of NEC associated with enteral feeding.

Target infection	Reference	Objective and study design	Result	Final outcome
		with enteral feeding. **Study Design:** An observational study included all premature infants admitted to Level III NICU, at less than 33 weeks gestational age. An EHM diet was recommended which eliminated all bovine-based artificial milk, including bovine-based fortifier through the study period.	where NEC occurred in two of 199 infants (1%) ($p = 0.009$).	
NEC, LOS	[133]	**Objective**: To examine the effect of human milk on morbidity, specifically necrotizing enterocolitis (NEC), late onset sepsis (LOS), retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD) and neurodevelopment in infants born ≤28 weeks' gestation. **Study Design:** Systematic review and meta-analysis where online databases were searched, and comparisons were grouped as follows: exclusive human milk (EHM) versus exclusive preterm formula (EPTF), any human milk (HM) versus EPTF, higher versus lower dose HM.	Human milk intake was associated with a clear protective effect against NEC, with a 4% reduction in incidence. Intake of exclusive human milk (EHM) in any volume was superior to intake of EPTF and the higher the dose the greater the protection.	Improving the intake of mother's own milk (MOM) and/or donor HM results in reducing the morbidity in this population.

Table 2.
Summary of some of the major studies focused on evaluating protective role of breast milk on outcome of various neonatal infections.

growth of *S. aureus* and *S. epidermidis*, two of the common pathogens involve in neonatal skin infections as well as cases of late-onset sepsis [134]. HBD-2 is the predominate defensin actively participating in reducing the incidence of respiratory infections and since preterm infants have lower levels of lung AMPs, they are unable to clear pathogens effectively [135]. Similarly, higher concentrations of HBD-2 appears to have a protective role once NEC pathology is established and in severe NEC. Low HBD-2 expression is a predisposing factor for developing NEC in preterm and low birth weight infants [136]. Low levels of defensins (HBD-1 and HBD-2) in preterm infants are associated with increased incidence of intestinal pathology and onset of NEC. Animal models have showed that depletion of Paneth cells rich in defensins followed by enteric infection in test animal resulted in a clinical picture akin to human NEC [137]. HBD-2 is directly involved in killing range of nosocomial pathogens (*E. coli, S. aureus, Klebsiella, Salmonella*) as showed by in vitro assays [138, 139].

Lactoferrin (LF) is a major contributor showing direct bactericidal action on range of neonatal pathogens. Murakami et al. [91] showed that 32 μM concentration of Lactoferrin was able to exhibit potent killing of 77% Group A Streptococcus (GAS), 40% *S. aureus* and 17% *E. coli*. Therefore, LF supplementation in VLBW infants is an ideal prophylactic treatment option for reducing the incidence of deaths and complication due to serious infections such as blood stream infections, infantile diarrhea, NEC in this population. These findings form the rationale for further exploiting the potential clinical utility of antimicrobial proteins and peptides in the prevention and treatment of infections in infants.

7. Preterm infants: a special case

Prematurity has been reported to be second most common cause of death in children under 5 years of age. Preterm infants are those born before 37 weeks of completed gestation, extremely preterm are those born at less than 28 weeks of completed gestation whereas very preterm refers to infants born between 28 and 32 weeks of gestation [140]. The incidence of premature birth and related deaths have been steadily increasing each year with an estimated 15 million babies born preterm [15]. Such infants are at increased risk of development of serious life threatening infections such as necrotizing enterocolitis (NEC), late onset sepsis (LOS), bloodstream infections, pneumonia and other complications.

Therefore, the challenge is to further improve the future of preterm infants with early protection to be given immediately after birth starting in the neonatal care units.

Maternal milk, a complex fluid with several bioactive proteins and peptides is beyond doubt the best option for the preterm infant [141]. Literature reveals that bioactive factors are found in much higher levels in preterm milk as compared to full-term milk and such factors are totally lacking in formula milk and significantly decreased in donated milk. This focuses on the use of human milk fortifiers containing the useful functional peptides as an ideal choice acting as a prophylactic supplement to protect the preterm infant where breastfeeding is unavailable or insufficient.

Preterm milk has higher protein and peptide levels as reported by past workers [75, 142]. Ferranti et al. [143] found more than 100 peptides originating by break-down of casein protein from mother who gave birth at 25 week gestation which is a case of extreme preterm delivery. Similarly, Armaforte et al. [144] also found via 2D-SDS page technique that low molecular weight casein fragments are over expressed in preterm milk as compared to term milk whereas intact αS1-casein and

ß-casein were present in low concentrations in preterm milk than term milk. Similarly, Dallas and co-workers [145] reported that protein breakdown was higher with higher level of plasmin activity seen in preterm milk. This indicates higher protein degradation and higher release of endogenous and antimicrobial peptides in preterm milk. These findings clearly indicate that antimicrobial peptides present in higher amounts act as a ready source to fight off infection at different levels for the wellbeing and early life protection of the preterm infants while the infants own protein digestion ability is not fully developed while compensating for the underdeveloped innate system of these vulnerable preterm infants.

Ronayne et al. [146] studied the level of lactoferrin in term and preterm milk and observed that the levels of lactoferrin remained constant in preterm group from eight post-partum day onwards while the levels showed a significant decline in term milk. This indicates that high levels are very much required in maintaining protective barrier against pathogens in preterm infants. Albenzio et al. [147] also found while studying lactoferrin levels from 28 mothers belonging to term and preterm cases that highest values of lactoferrin were detected in preterm infant maternal milk from infants with low weight (less than 1400 g). These findings again point towards use of tailored supplementation strategies of Lactoferrin in neonatal units and even after home discharge. The newly isolated endogenous peptides mentioned earlier in Section 4.4, i.e., PDC213 and ß-casein-197 have also been reported to be present in significantly higher concentrations in preterm milk as compared to term milk samples suggesting the role of breast milk naturally tailored to aid in giving maximum protection to such infants.

Human milk peptides also play an important role in preventing the onset of serious infections in VLBW infants. Meinzen-Derr et al. [129] studied the association between total human milk intake and the risk of developing necrotizing enterocolitis (NEC), a common cause of early death in extremely low birth infants. After, analysis of 1272 infants in a multicenter randomized, double-blind trial, results demonstrated protective effect of human milk intake with a dose dependent relationship seen between milk intake volumes and risk of developing NEC or death in such low birth weight infants. Similar cohort study also reported significantly lower rates of sepsis and death in low birth infants receiving less than 50 ml per kg per day of fortified human milk [148]. Sisk et al. [149] also reported six-fold decrease in the odds of NEC among 202 very low birth infants that received at least 50% human milk intake. Trend et al. [150] recently investigated the levels and antimicrobial activity of antimicrobial proteins and peptides in breast milk consumed by preterm infants, and whether deficiencies of these factors were associated with late-onset neonatal sepsis (LOS). They collected breast milk from mothers of preterm infants (32 weeks gestation) was collected on days 7 (n = 88) and 21 (n = 77) postpartum and concentrations of lactoferrin, LL-37, HBD-1 and HBD-2 were measured. Results indicated that levels of most AMPs and antibacterial activity in preterm breast milk were higher at day 7 than at day 21. The consumption of AMPs was significantly lower in preterm infants who subsequently developed LOS compared to matched controls. This highlights the need for research to improve upon the total feed intake, i.e., feeding tolerance, feeding volumes or increasing the total quantity of milk AMPs consumed through supplementation, that may be useful in reducing the risk of LOS in preterm infants.

7.1 Human milk fortification for preterm protection

Human milk is beyond doubt the best option to be given to the preterm. Human breast milk has many important proteins and peptides totally absent or present in low levels in bovine milk. For example, lysozyme is 1000 times highly concentrated

in human milk than cow's milk. Osteopontin which is essential in establishing immunity is 10 times more concentrated in human milk. Similarly, Lactoferrin is 20 times more concentrated in human milk and is an excellent antimicrobial agent along with bifidogenic properties [151]. As per WHO and American Academy of Pediatrics, pasteurized Human Donor Milk (HDM) is recommended as a second choice where mother's own milk is unavailable to the preterm infant [152]. However, pasteurization may reduce some of the important immune cells, bioactive proteins and functional peptides therefore research is now being focused on to fortify human milk to fulfill the need of the preterm infant. A randomized clinical trial reported that oral lactoferrin supplementation to preterm infants showed a decreased incidence of late-onset sepsis, a common cause of early deaths in such infants [153]. Enrichment of donated milk with essential disease fighting proteins and peptides to be given as soon as possible and until discharge from hospital is required for the preterm infant as recommended by European Society of Pediatric Gastroenterology, Hepatology and Nutrition [154]. Clinical study on bovine lactoferricin added to infant formula showed significant reduction in upper respiratory diseases in such infants aged 6–12 months of age [155].

Manzoni et al. [156] also reported a reduction in sepsis cases in premature infants which were given oral supplements of bovine lactoferricin. This opens a new window of use of supplementation of human lactoferrin based fortifiers acting as a therapy for the target infants.

Low birth weight infants may take very low volumes of mother's milk and due to these low volume intake, the levels of useful bioactive peptides may also be deficient in such infants. In such cases, human milk should be supplemented with external factors so that even low volumes carry enough of the protective peptides to meet the demand of the preterm and VLBW infants.

European milk Bank Association working group recognizes standard fortification as the most utilized regimen in NICU. It also encourages the concept of "Individualized Fortification" to optimize nutrient uptake further introducing the concept of "Adjustable Fortification" and "Target Fortification" to specifically modify the human milk to be administered to such infants [157]. However, we need to know more about the complexity of the various components of breast milk as there is a lot of variability in the levels and activity of different proteins and peptides from one mother to other and from one infant to other.

8. Breast milk in era of antibiotic resistance

Antimicrobial resistance is one of the leading global threat to public health worldwide. The VLBW and preterm infants admitted to NICU are at an increased risk of developing range of nosocomial infections and if such an infection is caused by a resistant strain, the situation becomes still worse to treat. Neonates are particularly at risk of exposure to resistant strains within the hospital environment that includes methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant *S. aureus* (VRSA), methicillin resistant *Staphylococcus epidermidis*, penicillin resistant strains of *Streptococcus pneumoniae*, extended spectrum beta-lactamase producing *E. coli*, *Klebsiella pneumoniae*, etc. [158–160].

Mortality is higher for children with drug-resistant infections, such as MRSA (a common skin and soft tissue infection) and infections caused by extended spectrum beta-lactamase-producing bacteria [161]. It is estimated that sepsis infections due to resistant strains accounts for approximately 214,000 neonatal deaths each year [162]. There is an urgent need for development of novel antibacterial drugs with unique mechanism of action that are not susceptible to existing resistance mechanisms being adopted by the bugs.

Human milk proteins and peptides offer a potent solution to fight life threatening infections especially in the infant population. It has been postulated by many workers that bacterial resistance to antimicrobial proteins and peptides is much less likely to evolve than the conventional antibiotics owing to their broad non-specific antibacterial mechanism of action that include membrane disruption and inhibition of cellular proteases [163, 164]. Also, most of these proteins and peptides work in a

Study	Milk peptide involved	Major findings
[166]	Ranalexin	Ranalexin (belonging to cathelicidin class) along with lysostaphin combination found to rapidly kill MRSA strain without affecting skin normal flora.
[167]	Indolicidin and Ranalexin	A group designed four hybrid peptides (Indolicidin and Ranalexin) that exhibited strong antibacterial activity against MRSA in vitro.
[168]	Indolicidin	Indolicidin—another family of peptides belonging to cathelicidin alone as well in combination with antibiotic was able to clear MRSA biofilm.
[169]	LL-13 and LL-17 (truncated forms of LL-37)	LL-37 and its truncated forms (LL-13 and LL-17) alone or in combination with vancomycin showed that both the truncated forms showed significant synergy and increased the susceptibility of VRSA to vancomycin. These two peptides also showed a strong ability to inhibit in vitro MRSA biofilm formation.
[170]	LL-37	An in vitro and in vivo study of Nafcillin (b-lactam drug) identified that it enhances killing of MRSA by increasing the binding of LL-37 to the MRSA membrane.
[171]	LL-37 and IDR-1	LL-37 and IDR-1 (innate defense regulator peptide) useful in ameliorating MRSA induced pneumonia using in vivo model of pneumonia in C57Bl/6 mice. The peptide combination was able to restore pulmonary function and decrease release of inflammatory cytokines in vivo through their immunomodulatory effect.
[172]	Recombinant hCAP18/ LL-37	Reported prokaryotic expression of full length hCAP18/LL-37 and its cathelin like prosequence. The workers showed that human cathelin like domain acts as a cysteine proteinase inhibitor and showed potent in vitro activity against resistant strains of *E. coli* and MRSA.
[173]	Human lactoferrin	Study reported that the bacteriostatic activity of human lactoferrin is solely due to its iron-chelating binding properties and is not influenced by antibiotic resistance of the pathogen involved. Therefore, therapeutic approach based on the use lactoferrin combined is a potent therapy against infections caused by resistant strains.
[174]	Lactoferrin derived HLR1r -synthetic	Reported a novel antimicrobial peptide structurally derived from human milk protein lactoferrin, HLR1r. They demonstrated potent activity against MRSA using in vivo model of pig infected with MRSA. The peptide also exhibited anti-inflammatory properties with significant reduction in inflammatory cytokines (TNF-α, IL-6 etc.)
[175]	Recombinant Lactoferrin (rLF)	Studied the efficacy of novel recombinant mouse lactoferrin to protect MRSA infection in a mouse model of peritonitis. The protein exhibited unique immunomodulatory mechanism in ameliorating the infection by decreasing the levels of inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-10) post lactoferrin treatment.

Table 3.
Summary of role of milk derived bioactive peptides activity against drug-resistant bacterial pathogens.

synergistic manner and thus work more efficiently in combination therapy. This also further decreases the frequency of developing of resistant mutants within the target population. Recent study at University of Helsinki examined 16 mother-infant pairs for antibiotic resistance patterns in the infant gut. The result showed that infants that are breastfed for at least 6 months have less antibiotic resistant bacteria in their gut as compared with babies fed for a shorter time [165]. The major studies focused on the evaluation of human milk derived proteins and/or peptides and their activity against resistant bacterial strains either in their purified form or in recombinant form are summarized in **Table 3**.

There is an urgent need for developing novel antibacterial drug with unique mechanisms of action that are not susceptible to existing resistant mechanisms. One such breakthrough discovery has been the purification of protein-lipid complex from human milk. Marks et al. [176] reported for the first time, the role of **HAMLET**, a novel protein-lipid complex purified from casein fraction of human milk. HAMLET stands for Human α-lactalbumin made lethal to tumor cells. This complex was able to kill both the resistant and sensitive strains of *Streptococcus pneumoniae*, leading cause of neonatal pneumonia and deaths [177]. Also, HAMLET complex when used along with common antibiotics (Penicillin, erythromycin, gentamycin) actually potentiated their effect as pneumococci was made more susceptible as demonstrated in vitro biofilm model and in vivo mice nasopharygeal colonization model. The complex also showed activity against resistant strains of *Haemophilus influenzae* and *Moraxella* spp. The protein complex binds to bacteria and stops the flow of ions in and out of the cells, it also blocks important enzymes required by the bacteria to obtain energy. This unique mechanism makes the bacterial cell weak and easily susceptible to damage by common antibiotics as HAMLET synergy reduced the dose of antibiotics by a factor of eight to kill MRSA, VRSA and *S. pneumoniae*. It was able to re-sensitize MRSA to methicillin and vancomycin intermediate *S. aureus* (VISA) to vancomycin by depolarization of bacterial membrane thus dissipating the Proton motive force leading to more access and easy binding of drug to target bacterial cell [178, 179].

9. Utility of human milk derived antimicrobial peptides and future implications

The potential role of various antimicrobial proteins and peptides present in breast milk against the large array of neonatal infections warrants serious future work towards decreasing neonatal morbidity and deaths by further exploiting their clinical utility.

Cellceutix Corporation has completed phase II trials of Brilacidin (synthetic analog of human defensin) in treating acute bacterial skin infections and preclinical testing against otitis media and ocular infections [78]. Lytix Biopharma has completed phase II trials of synthetic peptide, i.e., LTX-109 in impetigo (a problematic condition primarily affecting young children). Talactoferrin, a recombinant human LF, has been tested in a phase I study in preterm neonates [180]. Other AMPs, such as LF 1-11 (hLF1-11), have undergone safety and tolerability testing for delivery in healthy volunteers [181]. The use of recombinant congeners of AMPs represents an ideal treatment to improve circulating levels and thus improve outcomes from bacterial infection in infants especially in preterm infants. These peptides either induced endogenously or as exogenously administered congeners, may help prevent and treat infections in highly susceptible infants in early life. This is possible through production of recombinant human milk proteins in large quantities to be used for the manufacture of fortified infant formula for special cases. Ward et al.

[182] reported the production of commercial quantities of bioactive recombinant human lactoferrin in *Aspergillus awamori*. The technology combined with strain improvement program yielded high concentration of intact recombinant lactoferrin. Also, the use of transgenic animals as bioreactors for the synthesis of the recombinant proteins secreted into milk is worth exploring [183, 184]. With the advent of targeted genome editing technologies like CRISPR/Cas9 system, it has been possible to generate economically important animals that produce recombinant proteins in milk. Therefore, future strategies need to focus on isolation of unidentified novel milk peptides and their recombinant congeners for mass production that can be administered as supplementary feeds for prevention and treatment of the most devastating neonatal infections such as pneumonia, neonatal sepsis, diarrheal diseases, NEC, etc.

10. Conclusion

Human breast milk is the most powerful functional food known. It is a reservoir of bioactive proteins and peptides that contribute to the enormous health benefits of breast milk. Antimicrobial proteins and peptides present in breast milk confer protection against microbial insult. They play an active role against invasion by neonatal pathogens through direct and indirect mechanisms thus decreasing the associated neonatal morbidity and mortality. Further, these peptides are expressed at higher levels in preterm milk than term milk acting as ready source of protection for the preterm and VLBW infants. In the era of antibiotic resistance, the need to exploit these unique milk derived peptides become still more important as they represent an important alternative strategy to fight against drug resistant infections that is on the rise in the neonatal population as well. Research focusing on incorporation of novel antimicrobial proteins and peptides in formula feed or to be given along with donor milk may allow for providing early life protection to the preterm infants decreasing the incidence of premature deaths due to serious life threatening neonatal infections. The role of recombinant DNA technology for mass production of these novel antimicrobial peptides followed by their safety and efficacy trials warrants future work.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] The World Health Organization. Report of the Expert Consultation. The Optimal Duration of Exclusive Breast Feeding. Report Reference Number: WHO/NHD/01.09. Geneva, Switzerland. 2001. Available from: http://www.who.int/nutrition/publications/infantfeeding/WHO_NHD_01.09/en/ [Accessed: 30 January 2017]

[2] American Association of Pediatrics. AAP Reaffirms Breast Feeding Guidelines. 2012. Available from: https://www.aap.org/en-us/about-the-aap/aap-press-room/pages/aap-reaffirms-breast-feeding-guidelines.aspx [Accessed: 30 January 2017]

[3] Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, et al. Breastfeeding and child cognitive development: New evidence from a large randomized trial. Archives of General Psychiatry. 2008;**65**(5):578-584. DOI: 10.1001/ archpsyc.65.5.578

[4] Dieterich CM, Felice JP, O'Sullivan E, Rasmussen KM. Breastfeeding and health outcomes for the mother-infant dyad. Pediatric Clinics of North America. 2013;**60**(1): 31-48. DOI: 10.1016/j.pcl.2012.09.010

[5] O'Sullivan A, Farver M, Smilowitz JT. The influence of early infant-feeding practices on the intestinal microbiome and body composition in infants. Nutrition and Metabolic Insights. 2015;**8** (Suppl 1):1-9. DOI: 10.4137/NMI.S29530

[6] Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. Lancet. 2016;**387**(10017): 475-490. DOI: 10.1016/S0140-6736(15) 01024-7

[7] Gao X, McMahon RJ, Woo JG, Davidson BS, Morrow AL, Zhang Q. Temporal changes in milk proteomes reveal developing milk functions. Journal of Proteome Research. 2012; **11**(7):3897-3907. DOI: 10.1021/ pr3004002

[8] Ballard O, Morrow AL. Human milk composition: Nutrients and bioactive factors. Pediatric Clinics of North America. 2013;**60**(1):49-74. DOI: 10.1016/j.pcl.2012.10.002

[9] Lonnerdal B. Bioactive proteins in human milk: Mechanisms of action. The Journal of Pediatrics. 2010;**156**(2 Suppl): S26-S30. DOI: 10.1016/j.jpeds.2009.11.017

[10] Lonnerdal B. Human Milk: Bioactive proteins/peptides and functional properties. Nestle Nutrition Institute Workshop Series. 2016;**86**:97-107. DOI: 10.1159/000442729

[11] Zhu J, Dingess KA. The functional power of the human milk proteome. Nutrients. 2019;**11**(8):1834. DOI: 10.3390/nu11081834

[12] Cacho NT, Lawrence RM. Innate immunity and breast milk. Frontiers in Immunology. 2017;**8**:584. DOI: 10.3389/ fimmu.2017.00584

[13] Lonnerdal B. Bioactive proteins in breast milk. Journal of Paediatrics and Child Health. 2013;**49**:1-7. DOI: 10.1111/ jpc.12104

[14] Hakansson AP. Protective effects of human milk antimicrobial peptides against bacterial infection. The Journal of Pediatrics. 2015;**91**:4-5. DOI: 10.1016/ j.jped.2014.10.001

[15] Boquien CY. Human milk: An ideal food for nutrition of preterm newborn. Frontiers in Pediatrics. 2018;**6**:295. DOI: 10.3389/fped.2018.00295

[16] Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: A

review on its composition and bioactivity. Early Human Development. 2015;**91**:629-635. DOI: 10.1016/j.earlhumdev.2015.08.013

[17] Khaldi N, Vijayakumar V, Dallas DC, Guerrero A, Wickramasinghe S, Smilowitz JT, et al. Predicting the important enzymes in human breast milk digestion. Journal of Agricultural and Food Chemistry. 2014;**62**:7225-7232. DOI: 10.1021/jf405601e

[18] Lonnerdal B, Erdmann P, Thakkar SK, Sauser J, Destaillats F. Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: A developmental perspective. The Journal of Nutritional Biochemistry. 2017;**41**:1-11. DOI: 10.1016/j.jnutbio.2016.06.001

[19] Atkinson SA. Effects of gestational stage at delivery on human milk components. In: Jenson RG, editor. Handbook of Milk Composition. New York, NY, USA: Academic Press Inc.; 1995. pp. 222-236

[20] Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clinical Nutrition. 2011;**30**(2):215-220. DOI: 10.1016/j.clnu.2010.08.003

[21] Faerk J, Skafte L, Petersen S, Peitersen B, Michaelsen KF. Macronutrients in milk from mothers delivering preterm. Advances in Experimental Medicine and Biology. 2001;**501**:409-413. DOI: 10.1007/978-1-4615-1371-1_51

[22] Castellote C, Casillas R, Ramirez-Santana C, Perez-Cano FJ, Castell M, Moretones MG, et al. Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. Journal of Nutrition. 2011;**141**(6):1181-1187. DOI: 10.3945/jn.110.133652

[23] Thibeau SM, D'Apolito K. Review of the relationships between maternal characteristics and preterm breast milk immune components. Biological Research for Nursing. 2012;**14**(2):207-216. DOI: 10.1177/1099800411400064

[24] Anderson DM, Williams FH, Merkatz RB, Schulman PK, Kerr DS, Pittard WB 3rd. Length of gestation and nutritional composition of human milk. The American Journal of Clinical Nutrition. 1983;**37**(5):810-814. DOI: 10.1093/ajcn/37.5.810

[25] Chandra RK. Immunoglobulin and protein levels in breast milk produced by mothers of preterm infants. Nutrition Research. 1982;**2**:27-30. DOI: 10.1016/S0271-5317(82)80023-7

[26] Gross SJ, Buckley RH, Wakil SS, McAllister DC, David RJ, Faix RG. Elevated IgA concentration in milk produced by mothers delivered of preterm infants. The Journal of Pediatrics. 1981;**99**(3):389-393. DOI: 10.1016/s0022-3476(81)80323-x

[27] Atkinson SA, Bryan MH, Anderson GH. Human milk: Difference in nitrogen concentration in milk from mothers of term and premature infants. The Journal of Pediatrics. 1978;**93**:67-69. DOI: 10.1016/S0022-3476(78)80602-7

[28] Lemons JA, Moye L, Hall D, Simmons M. Differences in the composition of preterm and term human mass index, and parity number of lipid, protein, and secretory immunoglobulin A concentrations of human milk. Breastfeeding Medicine. 1982;**7**:179-188

[29] Anderson GH, Atkinson SA, Bryan MH. Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. The American Journal of Clinical Nutrition. 1981;**34**(2):258-265. DOI: 10.1093/ajcn/34.2.258

[30] Lucas A, Hudson GJ. Preterm milk as a source of protein for low birthweight infants. Archives of Disease in Childhood. 1984;**59**(9):831-836. DOI: 10.1136/adc.59.9.831

[31] Bachour P, Yafawi R, Jaber F, Choueiri E, Abdel-Razzak Z. Effects of smoking, mother's age, body mass index, and parity number on lipid, protein, and secretory immunoglobulin A concentrations of human milk. Breastfeeding Medicine. 2012; **7**(3):179-188. DOI: 10.1089/bfm.2011.0038

[32] Nommsen-Rivers LA, Dewey KG. Growth of breastfed infants. Breastfeeding Medicine. 2009;**4** (Suppl 1):S45-S49. DOI: 10.1089/bfm.2009.0048

[33] Ramirez-Santana C, Perez-Cano FJ, Audi C, Castell M, Moretones MG, Lopez-Sabater MC, et al. Effects of cooling and freezing storage on the stability of bioactive factors in human colostrum. Journal of Dairy Science. 2012;**95**(5):2319-2325. DOI: 10.3168/jds.2011-5066

[34] Sharma V, Sharma C, Chauhan S, Kaur S. Effect of lactation age and storage on the antibacterial potency of human breast milk against neonatal pathogens. Journal of Pure and Applied Microbiology. 2018;**12**(3):1307-1314. DOI: 10.22207/JPAM.12.3.33

[35] Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. The Journal of Allergy and Clinical Immunology. 2001;**108**(4):516-520. DOI: 10.1067/mai.2001.118130

[36] Evans TJ, Ryley HC, Neale LM, Dodge JA, Lewarne VM. Effect of storage and heat on antimicrobial proteins in human milk. Archives of Disease in Childhood. 1978;**53**(3): 239-241. DOI: 10.1136/adc.53.3.239

[37] Akinbi H, Meinzen-Derr J, Auer C, Ma Y, Pullum D, Kusano R, et al. Alterations in the host defense properties of human milk following prolonged storage or pasteurization. Journal of Pediatric Gastroenterology and Nutrition. 2010;**51**:347-352. DOI: 10.1097/MPG.0b013e3181e07f0a

[38] Chang JC, Chen CH, Fang LJ, Tsai CR, Chang YC, Wang TM. Influence of prolonged storage process, pasteurization, and heat treatment on biologically-active human milk proteins. Pediatrics and Neonatology. 2013;**54**(6): 360-366. DOI: 10.1016/j.pedneo.2013.03.018

[39] Lawrence RA, Lawrence RM. Breastfeeding: A Guide for the Medical Professions. 7th ed. Maryland Heights: Elsevier Mosby; 2011. p. 253

[40] Pandita A, Sharma D, Kumar C. Lactoferrin and its role in neonatology: A review article. Journal of Pediatrics & Neonatal Care. 2015;**2**:00062

[41] Bhimani RS, Vendrov Y, Furmanski P. Influence of lactoferrin feeding and injection against systemic staphylococcal infections in mice. Journal of Applied Microbiology. 1999; **86**(1):135-144. DOI: 10.1046/j.1365-2672.1999.00644.x

[42] Hendricks GM, Guo M. Bioactive components in human milk. In: Guo M, editor. Human Milk Biochemistry and Infant Formula Manufacturing. Cambridge, UK: Woodhead Publishing; 2014. pp. 33-54

[43] Farnaud S, Evans RW. Lactoferrin —A multifunctional protein with antimicrobial properties. Molecular Immunology. 2005;**40**(7):395-405. DOI: 10.1016/s0161-5890(03)00152-4

[44] Orsi N. The antimicrobial activity of lactoferrin: Current status and perspectives. BioMetals. 2004;**17**(3): 189-196. DOI: 10.1023/b:biom.0000027691.86757.e2

[45] Ellison RT III, Giehl TJ. Killing of gram-negative bacteria by lactoferrin and lysozyme. The Journal of Clinical Investigation. 1991;**88**:1080-1091

[46] Lonnerdal B, Iyer S. Lactoferrin: Molecular structure and biological function. Annual Review of Nutrition. 1995;**15**:93-110. DOI: 10.1146/annurev.nu.15.070195.000521

[47] Davidson LA, Donovan SM, Lonnerdal B, Atkinson SA. Excretion of human milk proteins by term and premature infants. In: Atkinson SA, Lonnerdal B, editors. Protein and Non-Protein Nitrogen in Human Milk. Boca Raton, FL: CRC Press; 1989. pp. 161-172

[48] Davidson GP. Passive protection against diarrheal disease. Journal of Pediatric Gastroenterology and Nutrition. 1996;**23**:207-212. DOI: 10.1097/00005176-199610000-00001

[49] Lawrence RA. Host-resistance factors and immunologic significance of human milk. Breastfeeding: A guide for the medical profession. Elsevier Health Sciences. 2005;**6**:171-214

[50] Goldblum RM. The role of IgA in local immune protection. Journal of Clinical Immunology. 1990;**10**:64

[51] Wold AE, Hanson LA. Defence factors in human milk. Current Opinion in Gastroenterology. 1994;**10**:652. DOI: 10.1016/s0022-3476(73)80453-6

[52] Delneri MT, Carbonare SB, Silva ML, Palmeira P, Carneiro-Sampaio MM. Inhibition of enteropathogenic *Escherichia coli* adhesion to HEp-2 cells by colostrum and milk from mothers delivering low-birth-weight neonates. European Journal of Pediatrics. 1997; **156**(6):493-498. DOI: 10.1007/s004310050646

[53] Haschke F, Haiden N, Thakkar SK. Nutritive and bioactive proteins in breastmilk. Annals of Nutrition & Metabolism. 2016;**69**(Suppl. 2):17-26. DOI: 10.1159/000452820

[54] Cuilliere ML, Trégoat V, Bene MC, Faure G, Montagne P. Changes in the kappa-casein and beta-casein concentrations in human milk during lactation. Journal of Clinical Laboratory Analysis. 1999;**13**(5):213-218. DOI: 10.1002/(SICI)1098-2825(1999)13:5<213::AID-JCLA4>3.0.CO;2-F

[55] Aniansson G, Andersson B, Lindstedt R, Svanborg C. Anti-adhesive activity of human casein against *Streptococcus pneumoniae* and *Haemophilus influenzae*. Microbial Pathogenesis. 1990;**8**:315-323. DOI: 10.1016/0882-4010(90)90090-d

[56] Newburg DS. Do the binding properties of oligosaccharides in milk protect human infants from gastrointestinal bacteria? The Journal of Nutrition. 1997;**127**:980S-984S. DOI: 10.1093/jn/127.5.980S

[57] Stromqvist M, Falk P, Bergstrom S, Hansson L, Lonnerdal B, Normark S, et al. Human milk kappa-casein and inhibition of *Helicobacter pylori* adhesion to human gastric mucosa. Journal of Pediatric Gastroenterology and Nutrition. 1995;**21**(3):288-296. DOI: 10.1097/00005176-199510000-00006

[58] Schack L, Lange A, Kelsen J, Agnholt J, Christensen B, Petersen TE, et al. Considerable variation in the concentration of osteopontin in human milk, bovine milk, and infant formulas. Journal of Dairy Science. 2009;**92**(11):5378-5385. DOI: 10.3168/jds.2009-2360

[59] Nagatomo T, Ohga S, Takada H, Nomura A, Hikino S, Imura M, et al. Microarray analysis of human milk cells: Persistent high expression of osteopontin during the lactation period. Clinical and Experimental Immunology. 2004;**138**(1):47-53

[60] Chatterton DEW, Rasmussen JT, Heegaard CW, Sørensen ES, Petersen TE. In vitro digestion of novel milk protein ingredients for use in infant formulas: Research on biological functions. Trends in Food Science and Technology. 2004;**15**:373-383. DOI: 10.1016/j.tifs.2003.12.004

[61] Wang KX, Denhardt DT. Osteopontin: Role in immune regulation and stress responses. Cytokine & Growth Factor Reviews. 2008;**19**: 333-345. DOI: 10.1016/j.cytogfr.2008.08.001

[62] Azuma N, Maeta A, Fukuchi K, Kanno C. A rapid method for purifying osteopontin from bovine milk and interaction between osteopontin and other milk proteins. International Dairy Journal. 2006;**16**:370-378. DOI: 10.1016/j.idairyj.2005.03.012

[63] Adkins Y, Lonnerdal B. Mechanisms of vitamin B12 absorption in breast-fed infants. Journal of Pediatric Gastroenterology and Nutrition. 2002; **35**:192-198. DOI: 10.1097/00005176-200208000-00016

[64] Adkins Y, Lonnerdal B. Potential host-defense role of a human milk vitamin B-12-binding protein, haptocorrin, in the gastrointestinal tract of breastfed infants, as assessed with porcine haptocorrin in vitro. The American Journal of Clinical Nutrition. 2003;**77**(5):1234-1240. DOI: 10.1093/ajcn/77.5.1234

[65] Shin K, Tomita M, Lonnerdal B. Identification of lactoperoxidase in mature human milk. The Journal of Nutritional Biochemistry. 2000;**11**: 94-102. DOI: 10.1016/s0955-2863(99)00082-0

[66] Kussendrager KD, van Hooijdonk AC. Lactoperoxidase: Physico-chemical properties, occurrence, mechanism of action and applications. The British Journal of Nutrition. 2000;**84**(Suppl 1):S19-S25. DOI: 10.1017/s0007114500002208

[67] Sharma S, Singh AK, Kaushik S, Sinha M, Singh RP, Sharma P, et al. Lactoperoxidase: Structural insights into the function, ligand binding and inhibition. International Journal of Biochemistry and Molecular Biology. 2013;**4**(3):108-128

[68] Sarr D, Toth E, Gingerich A, Rada B. Antimicrobial actions of dual oxidases and lactoperoxidase. Journal of Microbiology. 2018;**56**:373-386. DOI: 10.1007/s12275-018-7545-1

[69] Alexander DB, Iigo M, Yamauchi K, Suzui M, Tsuda H. Lactoferrin: An alternative view of its role in human biological fluids. Biochemistry and Cell Biology. 2012;**90**(3):279-306. DOI: 10.1139/o2012-013

[70] Lonnerdal B, Lien EL. Nutritional and physiologic significance of alpha-lactalbumin in infants. Nutrition Reviews. 2003;**61**:295-305. DOI: 10.1301/nr.2003.sept.295-305

[71] Permyakov EA, Berliner LJ. Alpha-lactalbumin: Structure and function. FEBS Letters. 2000;**473**:269-274. DOI: 10.1016/s0014-5793(00)01546-5

[72] Pellegrini A, Thomas U, Bramaz N, Hunziker P, von Fellenberg R. Isolation and identification of three bactericidal domains in the bovine α-lactalbumin molecule. Biochimica et Biophysica Acta. 1999;**1426**(3):439-448. DOI: 10.1016/s0304-4165(98)00165-2

[73] Liu B, Yu Z, Chen C, Kling DE, Newburg DS. Human milk mucin 1 and mucin 4 inhibit *Salmonella enterica* serovar Typhimurium invasion of human intestinal epithelial cells in vitro. The Journal of Nutrition. 2012;**142**(8): 1504-1509. DOI: 10.3945/jn.111.155614

[74] Newburg DS, Peterson JA, Ruiz-Palacios GM, Matson DO, Morrow AL,

Shults J, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet. 1998;**351**(9110):1160-1164. DOI: 10.1016/S0140-6736(97)10322-1

[75] Dallas DC, Guerrero A, Khaldi N, Borghese R, Bhandari A, Underwood MA, et al. A peptidomic analysis of human milk digestion in the infant stomach reveals protein-specific degradation patterns. The Journal of Nutrition. 2014;**144**(6):815-820. DOI: 10.3945/jn.113.185793

[76] Nielsen SD, Beverly RL, Underwood MA, Dallas DC. Release of functional peptides from mother's milk and fortifier proteins in the premature infant stomach. PLoS One. 2018;**13**(11): e0208204. DOI: 10.1371/journal.pone.0208204

[77] Hanson LA, Korotkova M. The role of breastfeeding in prevention of neonatal infection. Seminars in Neonatology. 2002;**7**(4):275-281. DOI: 10.1016/s1084-2756(02)90124-7

[78] Battersby AJ, Gibbons DL. The gut mucosal immune system in the neonatal period. Pediatric Allergy and Immunology. 2013;**24**(5):414-421. DOI: 10.1111/pai.12079

[79] Mohanty D, Jena R, Choudhury PK, Pattnaik R, Mohapatra S, Saini MR. Milk derived antimicrobial bioactive peptides: A review. International Journal of Food Properties. 2016;**19**: 837-846

[80] Wimley WC. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. ACS Chemical Biology. 2010; **5**(10):905-917. DOI: 10.1021/cb1001558

[81] Le CF, Fang CM, Sekaran SD. Intracellular targeting mechanisms by antimicrobial peptides. Antimicrobial Agents and Chemotherapy. 2017;**61**(4): e02340-16. DOI: 10.1128/AAC.02340-16

[82] Armogida SA, Yannaras NM, Melton AL, Srivastava MD. Identification and quantification of innate immune system mediators in human breast milk. Allergy and Asthma Proceedings. 2004;**25**:297-304

[83] Baricelli J, Rocafull MA, Vazquez D, Bastidas B, Baez-Ramirez E, Thomas LE. β-Defensin-2 in breast milk displays a broad antimicrobial activity against pathogenic bacteria. The Journal of Pediatrics. 2015;**91**:36-43. DOI: 10.1016/j.jped.2014.05.006

[84] Chu H, Pazgier M, Jung G, Nuccio SP, Castillo PA, de Jong MF, et al. Human α-defensin 6 promotes mucosal innate immunity through self-assembled peptide nanonets. Science. 2012;**337**(6093):477-481. DOI: 10.1126/science.1218831

[85] Jia HP, Starner T, Ackermann M, Kirby P, Tack BF, McCray PB Jr. Abundant human beta-defensin-1 expression in milk and mammary gland epithelium. The Journal of Pediatrics. 2001;**138**(1):109-112. DOI: 10.1067/mpd.2001.109375

[86] Tunzi CR, Harper PA, Bar-Oz B, Valore EV, Semple JL, Watson-MacDonell J, et al. Beta-defensin expression in human mammary gland epithelia. Pediatric Research. 2000; **48**(1):30-35. DOI: 10.1203/00006450-200007000-00008

[87] Raschig J, Mailander-Sanchez D, Berscheid A, Berger J, Strömstedt AA, Courth LF, et al. Ubiquitously expressed Human Beta Defensin 1 (hBD1) forms bacteria-entrapping nets in a redox dependent mode of action. PLoS Pathogens. 2017;**13**(3):e1006261. DOI: 10.1371/journal.ppat.1006261

[88] Yang D, Chertov O, Oppenheim JJ. The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity. Cellular and Molecular Life

Sciences. 2001;**58**:978-989. DOI: 10.1007/PL00000914

[89] Durr UH, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochimica et Biophysica Acta. 2006;**1758**(9):1408-1425. DOI: 10.1016/j.bbamem.2006.03.030

[90] Murakami M, Tonouchi H, Takahashi R, Kitazawa H, Kawai Y, Negishi H, et al. Structural analysis of a new anti-hypertensive peptide (beta-lactosin B) isolated from a commercial whey product. Journal of Dairy Science. 2004;**87**:1967-1974

[91] Murakami M, Dorschner RA, Stern LJ, Lin KH, Gallo RL. Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. Pediatric Research. 2005;**57**:10-15. DOI: 10.1203/01.PDR.0000148068.32201.50

[92] Boman HG. Antibacterial peptides: Basic facts and emerging concepts. Journal of Internal Medicine. 2003;**254**: 197-215. DOI: 10.1046/j.1365-2796.2003.01228.x

[93] Dorschner RA, Lin KH, Murakami M, Gallo RL. Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: Innate immunity during development of the adaptive response. Pediatric Research. 2003;**53**:566-572. DOI: 10.1203/01.PDR.0000057205.64451.B7

[94] Chen X, Niyonsaba F, Ushio H, Okuda D, Nagaoka I, Ikeda S, et al. Synergistic effect of antibacterial agents human beta-defensins, cathelicidin LL-37 and lysozyme against *Staphylococcus aureus* and *Escherichia coli*. Journal of Dermatological Science. 2005;**40**(2): 123-132. DOI: 10.1016/j.jdermsci.2005.03.014

[95] Scheid A, Li N, Jeffers C, Borriello F, Joshi S, Ozonoff A, et al. Antimicrobial peptide LL-37 and recombinant human mannose-binding lectin express distinct age- and pathogen-specific antimicrobial activity in human newborn cord blood in vitro. F1000Research. 2018;**7**:616. DOI: 10.12688/f1000research.14736.1

[96] Yekta MA, Verdonck F, Van Den Broeck W, Goddeeris BM, Cox E, Vanrompay D. Lactoferrin inhibits *E. coli* O157:H7 growth and attachment to intestinal epithelial cells. Veterinární Medicína. 2010;**55**(8):359-368

[97] Gifford JL, Hunter HN, Vogel HJ. Lactoferricin: A lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cellular and Molecular Life Sciences. 2005;**62**:2588-2598. DOI: 10.1007/s00018-005-5373-z

[98] Wakabayashi H, Teraguchi S, Tamura Y. Increased Staphylococcus-killing activity of an antimicrobial peptide, lactoferricin B, with minocycline and monoacylglycerol. Bioscience, Biotechnology, and Biochemistry. 2002;**66**:161-2167. DOI: 10.1271/bbb.66.2161

[99] Bruni N, Capucchio MT, Biasibetti E, Pessione E, Cirrincione S, Giraudo L, et al. Antimicrobial activity of lactoferrin-related peptides and applications in human and veterinary medicine. Molecules. 2016;**21**:E752. DOI: 10.3390/molecules21060752

[100] Brouwer CP, Rahman M, Welling MM. Discovery and development of a synthetic peptide derived from lactoferrin for clinical use. Peptides. 2011;**32**:1953-1963. DOI: 10.1016/j.peptides.2011.07.017

[101] Fu Y, Ji C, Chen X, Cui X, Wang X, Feng J, et al. Investigation into the antimicrobial action and mechanism of a novel endogenous peptide β-casein 197 from human milk. AMB Express. 2017; **7**(1):119. DOI: 10.1186/s13568-017-0409-y

[102] Sun Y, Zhou Y, Liu X, Zhang F, Yan L, Chen L, et al. Wang J antimicrobial activity and mechanism of PDC213, an endogenous peptide from human milk. Biochemical and Biophysical Research Communications. 2017;**484**(1):132-137. DOI: 10.1016/j.bbrc.2017.01.059

[103] WHO. Newborns: Reducing mortality. 2018. Retrieved from: http://www.who.int

[104] WHO Fact Sheet. 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/newborns-reducing-mortality

[105] Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: Evaluation of neonatal sepsis. Pediatric Clinics of North America. 2013;**60**(2):367-389. DOI: 10.1016/j.pcl.2012.12.003

[106] Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease–revised guidelines from CDC, 2010. MMWR - Recommendations and Reports. 2010; **59**:1-36

[107] Schrag SJ, Zywicki S, Farley MM, Reingold AL, Harrison LH, Lefkowitz LB, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. The New England Journal of Medicine. 2000;**342**:15-20. DOI: 10.1056/NEJM200001063420103

[108] Stoll BJ, Hansen NI, Sanchez PJ, Faix RG, Poindexter BB, Van Meurs KP, et al. Early onset neonatal sepsis: The burden of group B Streptococcal and *E. coli* disease continues. Pediatrics. 2011; **127**:817-826. DOI: 10.1542/peds.2010-2217

[109] Tsai CH, Chen YY, Wang KG, Chen CY, Chen CP. Characteristics of early-onset neonatal sepsis caused by *Escherichia coli*. Taiwanese Journal of Obstetrics & Gynecology. 2012;**51**: 26-30. DOI: 10.1016/j.tjog.2012.01.006

[110] Didier C, Streicher MP, Chognot D, Campagni R, Schnebelen A, Messer J, et al. Late-onset neonatal infections: Incidences and pathogens in the era of antenatal antibiotics. European Journal of Pediatrics. 2012;**171**:681-687. DOI: 10.1007/s00431-011-1639-7

[111] Lim WH, Lien R, Huang YC, Chiang MC, Fu RH, Chu SM, et al. Prevalence and pathogen distribution of neonatal sepsis among very-low-birth-weight infants. Pediatrics and Neonatology. 2012;**53**:228-234. DOI: 10.1016/j.pedneo.2012.06.003

[112] Shane AL, Hansen NI, Stoll BJ, Bell EF, Sánchez PJ, Shankaran S, et al. Methicillin-resistant and susceptible *Staphylococcus aureus* bacteremia and meningitis in preterm infants. Pediatrics. 2012;**129**:e914-e922. DOI: 10.1542/peds.2011-0966

[113] Thompson AM, Bizzarro MJ. Necrotizing enterocolitis in newborns: Pathogenesis, prevention and management. Drugs. 2008;**68**(9): 1227-1238. DOI: 10.2165/00003495-200868090-00004

[114] Coggins SA, Wynn JL, Weitkamp JH. Infectious causes of necrotizing enterocolitis. Clinics in Perinatology. 2015;**42**(1):133-154. DOI: 10.1016/j.clp.2014.10.012

[115] Goerens A, Lehnick D, Büttcher M, Daetwyler K, Fontana M, Genet P, et al. Neonatal ventilator associated pneumonia: A quality improvement initiative focusing on antimicrobial stewardship. Frontiers in Pediatrics. 2018;**24**(6):262. DOI: 10.3389/fped.2018.00262

[116] Bradley JS. Considerations unique to pediatrics for clinical trial design in hospital-acquired pneumonia and ventilator-associated pneumonia. Clinical

Infectious Diseases. 2010;**51**(Suppl 1): S136-S143. DOI: 10.1086/653063

[117] Cantey JB, Wozniak PS, Pruszynski JE, Sanchez PJ. Reducing unnecessary antibiotic use in the neonatal intensive care unit (SCOUT): A prospective interrupted time-series study. The Lancet Infectious Diseases. 2016;**16**:1178-1184. DOI: 10.1016/S1473-3099(16)30205-5

[118] Bhutta ZA, Black RE. Global maternal, newborn, and child health—So near and yet so far. The New England Journal of Medicine. 2013;**369**: 2226-2235. DOI: 10.1056/NEJMra1111853

[119] Turin CG, Ochoa TJ. The role of maternal breast milk in preventing infantile diarrhea in the developing world. Current Tropical Medicine Reports. 2014;**1**(2):97-105. DOI: 10.1007/s40475-014-0015-x

[120] Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A. Anti-infective properties of human milk. Journal of Nutrition. 2008;**138**(9):1801s-1806s. DOI: 10.1093/jn/138.9.1801S

[121] Lamberti LM, Fischer Walker CL, Noiman A, Victora C, Black RE. Breastfeeding and the risk for diarrhea morbidity and mortality. BMC Public Health. 2011;**11**:S15. DOI: 10.1186/1471-2458-11-S3-S15

[122] Li R, Dee D, Li CM, Hoffman HJ, Grummer-Strawn LM. Breastfeeding and risk of infections at 6 years. Pediatrics. 2014;**134**(Suppl 1):S13-S20. DOI: 10.1542/peds.2014-0646D

[123] Chamova R, Pancheva R, Dimitrova T, Bliznakova D. Protective effect of breast milk on urinary tract infection in children aged 0–3 years. Journal of IMAB - Annual Proceeding (Scientific Papers). 2018;**24**(1): 1918-1922. DOI: 10.5272/jimab.2018241.1918

[124] Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child under nutrition and overweight in low-income and middle-income countries. Lancet. 2013; **382**(9890):427-451. DOI: 10.1016/S0140-6736(13)60937-X

[125] Ashraf RN, Jalil F, Zaman S, Karlberg J, Khan SR, Lindblad BS, et al. Breast feeding and protection against neonatal sepsis in a high risk population. Archives of Disease in Childhood. 1991; **66**(4):488-490. DOI: 10.1136/adc.66.4.488

[126] Cesar JA, Victora CG, Barros FC, Santos IS, Flores JA. Impact of breast feeding on admission for pneumonia during post-neonatal period in Brazil: Nested case-control study. BMJ. 1999; **318**(7194):1316-1320. DOI: 10.1136/bmj.318.7194.1316

[127] Arifeen S, Black RE, Antelman G, Baqui A, Caulfield L, Becker S. Exclusive breastfeeding reduces acute respiratory infection and diarrhea deaths among infants in Dhaka slums. Pediatrics. 2001;**108**(4):E67. DOI: 10.1542/peds.108.4.e67

[128] Bachrach VR, Schwarz E, Bachrach LR. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: A meta-analysis. Archives of Pediatrics & Adolescent Medicine. 2003;**157**(3):237-243. DOI: 10.1001/archpedi.157.3.237

[129] Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF. Role of human milk in extremely low birth weight infants risk of necrotizing enterocolitis or death. Journal of Perinatology. 2009;**29**(1): 57-62. DOI: 10.1038/jp.2008.117

[130] Thibeau S, Boudreaux C. Exploring the use of mothers' own milk as oral care for mechanically ventilated very low-birth-weight preterm infants. Advances in Neonatal Care. 2013;**13**(3):

190-197. DOI: 10.1097/ANC.0b013e318285f8e2

[131] Ghaemi S, Navaei P, Rahimirad S, Behjati M, Kelishadi R. Evaluation of preventive effects of colostrum against neonatal conjunctivitis: A randomized clinical trial. Journal of Education Health Promotion. 2014;**23**(3):63. DOI: 10.4103/2277-9531.134776

[132] Herrmann K, Carroll K. An exclusively human milk diet reduces necrotizing enterocolitis. Breastfeeding Medicine. 2014;**9**(4):184-190. DOI: 10.1089/bfm.2013.0121

[133] Miller J, Tonkin E, Damarell RA, McPhee AJ, Suganuma M, Suganuma H, et al. A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants. Nutrients. 2018;**10**(6):707. DOI: 10.3390/nu10060707

[134] Nelson A, Hultenby K, Hell E, Riedel HM, Brismar H, Flock JI, et al. Staphylococcus epidermidis isolated from newborn infants express pilus-like structures and are inhibited by the cathelicidin-derived antimicrobial peptide LL37. Pediatric Research. 2009; **66**:174-178. DOI: 10.1203/PDR.0b013e3181a9d80c

[135] Starner TD, Agerberth B, Gudmundsson GH, McCray PB. Expression and activity of beta-defensins and LL-37 in the developing human lung. Journal of Immunology. 2005;**174**:1608-1615. DOI: 10.4049/jimmunol.174.3.1608

[136] Jenke ACW, Zilbauer M, Postberg J, Wirth S. Human β-defensin 2 expression in ELBW infants with severe necrotizing enterocolitis. Pediatric Research. 2012;**72**:513-520. DOI: 10.1038/pr.2012.110

[137] Salzman NH, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: A hypothesis on intimate interplay at the intestinal mucosa. Seminars in Immunology. 2007;**19**:70-83. DOI: 10.1016/j.smim.2007.04.002

[138] Tecle T, Tripathi S, Hartshorn KL. Review: Defensins and cathelicidins in lung immunity. Innate Immunity. 2010; **16**:151-159. DOI: 10.1177/1753425910365734

[139] Routsias JG, Karagounis P, Parvulesku G, Legakis NJ, Tsakris A. In vitro bactericidal activity of human beta-defensin 2 against nosocomial strains. Peptides. 2010;**31**:1654-1660. DOI: 10.1016/j.peptides.2010.06.010

[140] Guaraldi F, Salvatori G. Effect of breast and formula feeding on gut microbiota shaping in newborns. Frontiers in Cellular and Infection Microbiology. 2012;**2**:94. DOI: 10.3389/fcimb.2012.00094

[141] Gila-Diaz A, Arribas SM, Algara A, Martín-Cabrejas MA, López de Pablo ÁL, Saenz de Pipaon M, et al. A review of bioactive factors in human breast milk: A focus on prematurity. Nutrients. 2019;**11**(6):1307. DOI: 10.3390/nu11061307

[142] Zucht HD, Raida M, Adermann K, Magert HJ, Forssmann WG. Casocidin-I: A casein-alpha(s2) derived peptide exhibits antibacterial activity. FEBS Letters. 1995;**372**:185-188. DOI: 10.1016/0014-5793(95)00974-e

[143] Ferranti P, Traisci MV, Picariello G, Nasi A, Boschi V, Siervo M, et al. Casein proteolysis in human milk: Tracing the pattern of casein breakdown and the formation of potential bioactive peptides. The Journal of Dairy Research. 2004;**71**:74-87. DOI: 10.1017/s0022029903006599

[144] Armaforte E, Curran E, Huppertz T, Ryan A, Caboni MF, O'Connor PM, et al. Proteins and proteolysis in pre-term and term human

milk and possible implications for infant formulae. International Dairy Journal. 2010;**20**:715-723. DOI: 10.1016/j.idairyj.2010.03.008

[145] Dallas DC, Smink CJ, Robinson RC, Tian T, Guerrero A, Parker EA, et al. Endogenous human milk peptide release is greater after preterm birth than term birth. The Journal of Nutrition. 2015; **145**:425-433. DOI: 10.3945/jn.114.203646

[146] Ronayne de Ferrer PA, Baroni A, Sambucetti ME, López NE, CerianiCernadas JM. Lactoferrin levels in term and preterm milk. Journal of the American College of Nutrition. 2000;**19**: 370-373. DOI: 10.1080/07315724.2000.10718933

[147] Albenzio M, Santillo A, Stolfi I, Manzoni P, Iliceto A, Rinaldi M, et al. Lactoferrin levels in human milk after preterm and term delivery. American Journal of Perinatology. 2016;**33**(11): 1085-1089. DOI: 10.1055/s-0036-1586105

[148] Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: Beneficial outcomes of feeding fortified human milk versus preterm formula. Pediatrics. 1999; **103**(6):1150-1157. DOI: 10.1542/peds.103.6.1150

[149] Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM. Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. Journal of Perinatology. 2007;**27**(7):428-433. DOI: 10.1038/sj.jp.7211758

[150] Trend S, Strunk T, Hibbert J, Kok CH, Zhang G, Doherty DA, et al. Antimicrobial protein and peptide concentrations and activity in human breast milk consumed by preterm infants at risk of late-onset neonatal sepsis. PLoS One. 2015;**10**:e0117038. DOI: 10.1371/journal.pone.0117038

[151] Demmelmair H, Prell C, Timby N, Lonnerdal B. Benefits of lactoferrin, osteopontin and milk fat globule membranes for infants. Nutrients. 2017; **9**:817. DOI: 10.3390/nu9080817

[152] Bertino E, Giuliani F, Baricco M, Di Nicola P, Peila C, Vassia C, et al. Benefits of donor milk in the feeding of preterm infants. Early Human Development. 2013;**89**(Suppl 2):S3-S6. DOI: 10.1016/j.earlhumdev.2013.07.008

[153] Underwood MA. Human milk for the premature infant. Pediatric Clinics of North America. 2013;**60**(1):189-207. DOI: 10.1016/j.pcl.2012.09.008

[154] Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: Commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. Journal of Pediatric Gastroenterology and Nutrition. 2010;**50**(1):85-91. DOI: 10.1097/MPG.0b013e3181adaee0

[155] King JC Jr, Cummings GE, Guo N, Trivedi L, Readmond BX, Keane V, et al. A double-blind, placebo-controlled, pilot study of bovine lactoferrin supplementation in bottle-fed infants. Journal of Pediatric Gastroenterology and Nutrition. 2007;**44**:245-251. DOI: 10.1097/01.mpg.0000243435.54958.68

[156] Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: A randomized trial. Journal of the American Medical Association. 2009; **302**:1421-1428. DOI: 10.1001/jama.2009.1403

[157] Arslanoglu S, Boquien CY, King C, Lamireau D, Tonetto P, Barnett D, et al. Fortification of human milk for preterm infants: Update and recommendations of the European Milk Bank Association

(EMBA) Working Group on Human Milk Fortification. Frontiers in Pediatrics. 2019; 7:76. DOI: 10.3389/fped.2019.00076

[158] Bizzarro MJ, Gallagher PG. Antibiotic-resistant organisms in the neonatal intensive care unit. Seminars in Perinatology. 2007;**31**(1):26-32. DOI: 10.1053/j.semperi.2007.01.004

[159] Borchardt SM, DeBusscher JH, Tallman PA, Manning SD, Marrs CF, Kurzynski TA, et al. Frequency of antimicrobial resistance among invasive and colonizing group B streptococcal isolates. BMC Infectious Diseases. 2006; **6**:57. DOI: 10.1186/1471-2334-6-57

[160] Alarcon A, Pena P, Salas S, Sancha M, Omenaca F. Neonatal early onset *Escherichia coli* sepsis: Trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. The Pediatric Infectious Disease Journal. 2004;**23**:295-299. DOI: 10.1097/00006454-200404000-00004

[161] Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatrics. 2010;**10**:39. DOI: 10.1186/ 1471-2431-10-39

[162] Gandra S, Klein EY, Pant S, Malhotra-Kumar S, Laxminarayan R. Faropenem consumption is increasing in India. Clinical Infectious Diseases. 2016; **62**(8):1050-1052. DOI: 10.1093/cid/ ciw055

[163] Nuti R, Goud NS, Saraswati AP, Alvala R, Alvala M. Antimicrobial peptides: A promising therapeutic strategy in tackling antimicrobial resistance. Current Medicinal Chemistry. 2017;**24**(38):4303-4314. DOI: 10.2174/ 0929867324666170815102441

[164] Lei J, Sun L, Huang S, Zhu C, Li P, He J, et al. The antimicrobial peptides and their potential clinical applications. American Journal of Translational Research. 2019;**11**(7):3919-3931

[165] Parnanen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, et al. Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nature Communications. 2018;**9**(1):3891. DOI: 10.1038/ s41467-018-06393-w

[166] Desbois AP, Lang S, Gemmell CG, Coote PJ. Surface disinfection properties of the combination of an antimicrobial peptide, ranalexin, with an endopeptidase, lysostaphin, against methicillin-resistant *Staphylococcus aureus* (MRSA). Journal of Applied Microbiology. 2010;**108**:723-730. DOI: 10.1111/j.1365-2672. 2009.04472.x

[167] Jindal HM, Le C-F, MohdYusof MY, Velayuthan RD, Lee VS, Zain SM, et al. Antimicrobial activity of novel synthetic peptides derived from indolicidin and ranalexin against *Streptococcus pneumoniae*. PLoS One. 2015;**10**:e0128532. DOI: 10.1371/ journal.pone.0128532

[168] Dosler S, Mataraci E. In vitro pharmacokinetics of antimicrobial cationic peptides alone and in combination with antibiotics against methicillin resistant *Staphylococcus aureus* biofilms. Peptides. 2013;**49**:53-58. DOI: 10.1016/j. peptides.2013.08.008

[169] Shurko JF, Galega RS, Li C, Lee GC. Evaluation of LL-37 antimicrobial peptide derivatives alone and in combination with vancomycin against *S. aureus*. Journal of Antibiotics (Tokyo). 2018;**71**(11):971-974. DOI: 10.1038/ s41429-018-0090-7

[170] Le C-F, Yusof MYM, Hassan MAA, Lee VS, Isa DM, Sekaran SD. In vivo efficacy and molecular docking of designed peptide that exhibits potent anti-pneumococcal activity and

synergizes in combination with penicillin. Scientific Reports. 2015;**5**: 11886. DOI: 10.1038/srep11886

[171] Hou M, Zhang N, Yang J, Meng X, Yang R, Li J, et al. Antimicrobial peptide LL-37 and IDR-1 ameliorate MRSA pneumonia in vivo. Cellular Physiology and Biochemistry. 2013;**32**(3):614-623. DOI: 10.1159/000354465

[172] Zaiou M, Nizet V, Gallo RL. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. The Journal of Investigative Dermatology. 2003;**120**:810-816. DOI: 10.1046/j.1523-1747.2003.12132.x

[173] Aguila A, Herrera AG, Morrison D, Cosgrove B, Perojo A, Montesinos I, et al. Bacteriostatic activity of human lactoferrin against *Staphylococcus aureus* is a function of its iron-binding properties and is not influenced by antibiotic resistance. FEMS Immunology and Medical Microbiology. 2001;**31**:145-152. DOI: 10.1111/j.1574-695X.2001.tb00511.x

[174] Bjorn C, Mahlapuu M, Mattsby-Baltzer I, Hakansson J. Anti-infective efficacy of the lactoferrin-derived antimicrobial peptide HLR1r. Peptides. 2016;**81**:21-28. DOI: 10.1016/j.peptides.2016.04.005

[175] Hwang SA, Kruzel ML, Actor JK. Immunomodulatory effects of recombinant lactoferrin during MRSA infection. International Immunopharmacology. 2014;**20**(1): 157-163. DOI: 10.1016/j.intimp.2014.02.029

[176] Marks LR, Clementi EA, Hakansson AP. The human milk protein-lipid complex HAMLET sensitizes bacterial pathogens to traditional antimicrobial agents. PLoS One. 2012;**7**(8):e43514. DOI: 10.1371/journal.pone.0043514

[177] Alamiri F, Riesbeck K, Hakansson AP. HAMLET, a protein complex from human milk has bactericidal activity and enhances the activity of antibiotics against pathogenic *Streptococci*. Antimicrobial Agents and Chemotherapy. 2019;**63**:e01193-19. DOI: 10.1128/AAC.01193-19

[178] Marks LR, Clementi EA, Hakansson AP. Sensitization of *Staphylococcus aureus* to methicillin and other antibiotics in vitro and in vivo in the presence of HAMLET. PLoS One. 2013;**8**(5):e63158. DOI: 10.1371/journal.pone.0063158

[179] Sherman MP. Lactoferrin and necrotizing enterocolitis. Clinics in Perinatology. 2013;**240**:79-91. DOI: 10.1016/j.clp.2012.12.006

[180] Guntupalli K, Dean N, Morris PE, Bandi V, Margolis B, Rivers E, et al. A phase 2 randomized, double-blind, placebo-controlled study of the safety and efficacy of talactoferrin in patients with severe sepsis. Critical Care Medicine. 2013;**41**:706-716. DOI: 10.1097/CCM.0b013e3182741551

[181] Velden WJ, van Iersel TM, Blijlevens NM, Donnelly JP. Safety and tolerability of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11). BMC Medicine. 2009;**7**:-44. DOI: 10.1186/1741-7015-7-44

[182] Ward PP, Piddington CS, Cunningham GA, Zhou X, Wyatt RD, Conneely OM. A system for production of commercial quantities of human lactoferrin: A broad spectrum natural antibiotic. Biotechnology (NY). 1995; **13**(5):498-503. DOI: 10.1038/nbt0595-498

[183] Monzani PS, Adona PR, Ohashi OM, Meirelles FV, Wheeler MB. Transgenic bovine as bioreactors: Challenges and perspectives. Bioengineered. 2016;**7**:123-131. DOI: 10.1080/21655979.2016.1171429

[184] Shepelev MV, Kalinichenko SV, Deykin AV, Korobko IV. Production of recombinant proteins in the milk of transgenic animals: Current state and prospects. Acta Naturae. 2018;**10**(3): 40-47