2. REVIEW OF LITERATURE

2.1 GASTROINTESTINAL TRACT (GIT)

The gastrointestinal tract is a complex organ system whose primary functions are to carry out digestion and absorption of ingested nutrients and simultaneously to protect the body from ingested micro-organisms and noxious substances. After ingestion, food is mechanically divided, mixed with digestive enzymes and cleaved into readily absorbable particles. Nutrients are then absorbed through specialized mucosa and non-absorbed substances are expelled. More than 95% of ingested carbohydrate, fat and protein is usually absorbed during passage through a normally functioning gastrointestinal tract, although absorption of certain vitamins, minerals and trace elements may be less efficient (Robert et al., 1979).

2.2 STRUCTURE AND DEVELOPMENT OF THE GASTROINTESTINAL TRACT

Embryology

The primitive gut forms during the fourth week of gestation from the foregut, midgut and hindgut. The foregut gives rise to the pharynx, oesophagus, stomach, duodenum, liver, biliary apparatus and the pancreas.

The derivatives of the midgut are the small intestine (below the opening of the common bile duct) the caecum, the appendix, the ascending colon and the proximal part of the transverse colon.

The hindgut begins at the distal part of the transverse colon, the rectum and the superior portion of the anal canal.
2.3 FUNCTIONS OF THE GASTROINTESTINAL TRACT

After ingestion, the food is chewed and swallowed. Integrated mobility and secretion subsequently enhance digestion and mucosal contact for ultimate absorption. Secretion and mobility are subject to exogenous influences.

Normal Intestinal microflora

The indigenous microflora of the gastrointestinal (GI) tract in new-born humans and animals does not appear spontaneously. The foetus is sterile but it becomes contaminated with different microorganisms during birth. These microorganisms are then selected over time as changes occur in the GI tract. The different microorganisms inhabit different parts of the GI tract and then become characteristic of that particular habitat. Most of the microorganisms are thus eliminated such that at the end, the GI tract of infants is dominated by lactic acid bacteria and coliforms. Further changes in the population of the microflora occur during weaning, ending with the majority of the microbes being obligate anaerobes (Berg, 1996).

The microorganisms become distributed throughout the GI tract. Different parts of the GI tract become colonized by various populations of microorganisms. The stomach has a less dense microbial population which contains less than 100 cfu/ml of contents, its population is dominated by aerobic Gram positive organisms. The small intestine separates the less populated stomach from the densely populated colon. The microflora of the small intestine is similar to that of the stomach but has higher numbers of microorganisms. The most densely populated part of the GI tract (and of the whole body) is the large intestine (colon) (Berg, 1996). At least 500 species of anaerobic and facultative microbes, consisting among others several species of lactobacilli and bifidobacteria, are
found in the contents (Wolfram, 1999). Lactobacilli population comprising of about 60 species is found mainly in the small intestine while bifidobacteria are found in the colon.

The microflora depends on dietary residues in the GI tract for their metabolism and has the potential to influence processes in the colon. A new microbial ecosystem is born almost immediately after a human being is born. The sources of the microbes that make up the ecosystem varies from one infant to another and the composition of the ecosystem influences human physiology, development and disease. The distinct features of each baby’s microbial community are recognisable for intervals of weeks to months. The infant GI microbiota is more variable in its composition and less stable. Infant intestinal tract progresses from sterility to extremely dense colonization. Bifidobacteria almost always dominate the GI microbiota of breastfed infants by several weeks of age. Aerobic bacteria dominate the GI of formula fed infants(Palmer et al., 2007).

The microorganisms in the intestines can be either beneficial or detrimental. This division is dependent on whether the various enzymes produced by these microorganisms perform functions that harm or support the host. The overall health of an individual depends on the balance between the beneficial and detrimental effects of the intestinal microflora (Percival, 1997). The major physiological functions of the gut microflora may be summarised as follows (Holzapfel & Schillinger, 2002):

- barrier function
- immune system stimulation
- maintenance of mucosa nutrition and circulation
- production of nutrient/improved bioavailability
- stimulation of bowel motility
The importance of indigenous microflora as a natural resistance factor against potential pathogenic microorganisms was originally recognised in the 19th century by Metchnikoff. However, research interest on the use of bacteria for therapy disappeared in the 1940s and then re-emerged around the 1960s due to increased interest of people in natural ways of promoting health. The demand for natural products such as fermented foods and bacterial cultures for alleviating diarrhoea has stimulated the industry to conduct research into new products (Havenaar & Huis Veld, 1992).

The indigenous microflora can be influenced by factors both in the internal and external environments, resulting in an imbalance between beneficial and detrimental microbes. External factors include lack of food and water, travelling, use of antibiotics, and drugs for treatment of tumours and radiation (Luchansky et al., 1999). Other factors include peristalsis disorders, surgical operations of the stomach or small intestine, liver or kidney diseases, anaemia, immune disorders, emotional stress, poor diet and ageing. Loss of indigenous microflora due to disturbances by one or more of these factors favour predominance of microflora by harmful bacteria and also result in availability of empty adhesion sites on the intestinal epithelium. These empty sites may be occupied by any organisms including transient pathogenic microorganisms (Mitsuoka, 1996; Luchansky et al., 1999).

The confluence of food security, child care practices maternal health and education, sanitation and health services and social, political and economic structures act to influence the relationship between infant feeding and infant health (UNICEF, 1998).
2.4 DIARRHOEA

Diarrhoea, defined as an increase in the number of stools or a decrease in their consistency, is the major clinical manifestation of alterations of water and electrolyte transport by the alimentary tract. It is the result of disorders involving digestive, absorptive and secretary functions.

Diarrhoea is one of the most common symptoms of illness in paediatric practice. It usually appears in the form of acute diarrhoea, but it may also progress to a chronic state (Branski, 1984).

Escherich in 1885 identified a bacterium in stools of breast fed infants. It was later found in the intestinal tract of human beings and many higher vertebrates. The organism was designated *Escherichia coli* and was characterised as a plump, Gram negative, non-spore forming rod, motile by peritrichous flagella.

Pathogenic bacteria account for only about 30% of diarrhoea in children (Cramblett & Siewers, 1965; Connor & Barrott, 1967; Moffet *et al.*, 1968). The most important are enteropathogenic *E. coli*, *Shigella spp.* and *Salmonella spp.*

Diarrhoeal disease is an important cause of morbidity and mortality in children less than five years of age in tropical developing countries (Walsh & Warron, 1979).

An infections agent can be isolated from most children with acute diarrhoeal disease and more than one potential pathogen can be isolated in 15 - 30% of infants with diarrhoea (Black *et al.*, 1982 and Guerrant *et al.*, 1986).

Diarrhoea involves increased loss of fluid and electrolytes from the intestine. Bacterial aetiological agents continued to play a significant role (69.6%)
in diarrhoeal diseases. Enteroaggregative *E. coli* was common in the age group between 25-36 months, Shigellosis in 37-60 months and *Salmonella typhimurium* enteritis in 7-12 months of age. The other pathogens isolated were *Vibrio cholerae* (4.98%), species of *Aeromonas* (15.92%) (Ballal *et al.*, 2002).

The role of enteric pathogens in infants with persistent diarrhoea in Bedouin infants from Southern Israel were followed from birth to 18-23 months. Stool samples were obtained and feeding practices and history of diarrhoea were determined. In multivariate analysis age at first diarrhoeal illness and maternal age and education were independently and significantly associated with risk of persistent diarrhoea. These data suggested that persistent diarrhoea is a clinical entity that may be related less to a specific enteric pathogen and more to the health experiences of children and their home environment (Fraser *et al.*, 1998).

2.5 INFANTILE DIARRHOEA

Infantile diarrhoea is a main killer in infants. Factors affecting the frequencies of infantile diarrhoea include feeding practices A bi-monthly magazine "Health and Consumers" published in October 2004, disclosed that in Pakistan, 3500 infants die in a month due to diarrhoea mainly caused by consuming powder milk. In all, a total of 77,000 infants died so far in the country due to non-implementation of infant protection and Child Nutrition Ordinance 2002. Around the world, diarrhoea remains one of the most common illnesses among children. Diarrhoea kills about 4 million people in developing countries as well.

371 diarrhoeal children reporting to four hospitals in the district of Kurunegala, Sri Lanka were investigated for enteric pathogens between March and July 1987. 53.7% of the diarrhoeal children had one or more enteric pathogens.
*Shigella* species was the second most frequently isolated pathogens and the single most common cause of diarrhoea in children over two years of age. *Salmonella* species was isolated from 7.5% of diarrhoeal children. Other pathogens had prevalence rates of less than 5%. In view of the different transmission routes and characteristics of important pathogens in the study, a single environmental intervention is unlikely to solve public health problem posed by diarrhoeal diseases in Sri Lanka (Mertens *et al*., 1990).

1207 rectal swab specimens of children aged 1-35 months with acute watery diarrhoea presenting at a large diarrhoeal diseases hospital in urban Bangladesh were examined. *Escherichia coli* isolated was 15% followed by *Vibrio cholerae* 7%, other *Vibrios* were 9% and *Shigella* 4% and *Salmonella* <1%. These children can be managed effectively with oral rehydration therapy and proper feeding knowledge of pathogens associated with more severe forms of diarrhoea may help to optimise strategies for vaccination when suitable vaccines are available against enteric infections (Hoque *et al*., 1994).

Diarrhoea is common in all age groups but more common in infants. Annually at least 1500 million episodes of diarrhoea occur in children under age of 5 years. A prospective study was undertaken with 254 infants and children admitted with gastroenteritis to the Children's Hospital, Riyadh, Saudi Arabia during the period of September, 1980 to February, 1981. Analysis of data showed that 80% of the admissions were less than one year of age. The peak incidence occurred in the 0-5 months age group and 65.4% were on bottle feeding. The incidence of second and third degree malnutrition was 38.9% Marasmus was the commonest type of severe malnutrition seen. Hyper nataeemic dehydration occurred in 12.6% of the cases. The parasitic and bacterial isolation rate was 23.8%, *Salmonella* and Enteropathogenic *E. coli* being the commonest organisms.
The mortality rate was 9.1% and was higher among malnourished patients, those with hyper natraemia and in the younger age groups (Karrar et al., 1982).

A rapid and practical way of detecting diarrhoegenic E. coli is PCR which depends on detection of virulence factors. 36.8% of the E. coli isolates were identified as EPEC by serogrouping. 73% EPEC isolates were from below 2 years of age. High resistance to Ampicillin, Nalidixic acid, Co-trimoxazole, Ciprofloxacin and Norfloxacin was observed. 38.8% of E. coli were EAEC and no other diarrhoegenic Escherichia coli group was found by molecular characterisation.

Multidrug resistant EPEC strains are a common phenomenon in recent research. EPEC is still an important paediatric diarrheal pathogen. Out of the 100 stool samples from acute diarrheal cases in children below 5 years, the isolation of Escherichia coli was 82(82%), Klebsiella8(8%), Proteus5(5%), Citrobacter3(3%) and Enterococci 2(2%). Dehydration can be considered as one of the clinical features of paediatric diarrhoeas (Gandham Pavani Tilak et al., 2012).

Bacterial pathogens isolated from 210 paediatric patients admitted with acute diarrhoea to a regional hospital in the south western region of Saudi Arabia were Shigella (17.1%) followed by Salmonella (10.5%) and Enteropathogenic E. coli (EPEC)(3.8%) (Al-Jurayyan et al.,1994).

The enormous infantile death toll can be prevented by promotion of breast feeding, adoption of simple hygienic measures and standard case management.

In a therapeutic trial of hospitalised gastroenteritis infants previously on formula feeds, original feed or a lactose free soya preparation , previously on mother’s milk with or without a supplement, continued breastfeeding reduces the
duration of acute diarrhoea. The study concluded that (a) breastfeeding should be continued during an infantile diarrhoeal episode (b) soya preparations should not be used (Haffejee, 1990).

In a prospective study of infants and feeding in South East Queensland, Australia reported incidences of breast, bottle and mixed (breast and bottle) fed infants compared from birth to 1 year. Less diarrhoea was seen in 0-6 months old infants who were breastfed than those given bottle feed only. Breastfeeding protects against the incidence of diarrhoea. Bottle fed infants in upper social class families had less episodes of diarrhoea than lower social class families. Breastfeeding protects the infant diarrhoea and vomiting up to 6 months (Eaton-evans et al., 1987).

In a retrospective study of infant feeding among 257 Pima Indian women in Arizona, risk of developing diarrhoea was studied based on exclusively breastfed and other modes of feeding groups. Increased breastfeeding was associated with decreased rate of diarrhoea. Exclusive breastfeeding reduces the risk of episodes of gastroenteritis in less developed communities (Forman et al.,1984).

A serious concern has been raised by wide spread use of formula feeding. A WHO study revealed risk of diarrhoea for formula fed babies in developing nations averaging more than 6 times that of breast feeding babies.

Formula - fed feeding is associated with GIT infections (Myers et al., 1984).

An enormous and still growing body of medical research demonstrates that breast feeding is optimal choice for baby feeding (Khan et al., 2003).
It is only recently that modern scientific research has brought to light the paramount importance of breast feeding to the health of child.

In a study conducted in Pakistan, out of 100 cases of breast fed and bottle fed infants 84% were having repeated episodes of diarrhoea in formula feeding as compared to breast feed infants in which only 16% cases were have repeated the episodes of diarrhoea. It was found that diarrhoea was more common in rural areas, 60% in rural children compared to 40% among urban (Muhammad Hussain Khan et al., 2004).

A microbiological invesigation of acute summer gastroenteritis in Black South African infants by 81% of the infants revealed entero pathogenic agents. Enterotoxigenic bacteria were isolated from 15 patients (41%) (Barry et al.,1977). Breastfed infants decreased from 88% at 1 month to 29% at 12 months of age. No significant relationship was found between 4 categories( gastroenteritis, upper respiratory tract, otitis media and lower respiratory illness and type of infant feeding (Rubin et al.,1990).

The relationship between infectious illnesses and infant feeding was investigated in developing countries. 500 infants born in Copenhagen, Denmark were studied prospectively for first 12 months focussing on feeding practices and illnesses. Completely

A case control study by conducted in Brazil to study infant feeding and death due to diarrhoea reveals the association between infant feeding habits and infant mortality from diarrhoea. A population based case control study was conducted in two urban area in southern Brazil during 1985 (Cesar et al.,1985).
2.6 BREAST - FEEDING AND THE USE OF HUMAN MILK

**Human milk** is assumed to be the ideal food for the infant up to the age of 5 or 6 months ensuring optimal growth and development (ESPGAN,1982). In many respects, the most natural food available is human milk and it is unique.

Extensive research, especially in recent years, documents diverse and compelling advantages to infants from breast feeding and the use of human milk for infant feeding.

WHO recommends only breast milk for 6 months. If a baby is not breastfed, formula is required for the first year of life. Many mothers are not fully aware of the risks of formula feeding and so are making a choice without the necessary information.

Breast milk has hormones, growth factors, cytokines, immunocompetent cells and many biological properties. Breast milk composition is influenced by postnatal and gestational age. Cognitive development is enhanced by breastfeeding. Breastfeeding is lowest in France in Europe. Exclusive breastfeeding for 3 months shows lower incidence and severity of diarrhoea. Very few medications contraindicate breastfeeding.(Turck et al., 2013).

Ellestad-Sayed *et al.* (1979) in a retrospective study undertaken at two isolated Manitoba Indian communities to determine the relationship between infant feeding and infection during the first year of life revealed that out of the 158 infants, 28 were fully breastfed, 58 initially breastfed and then bottle fed and 72 were fully bottle fed. 10 times or more hospitalisation was seen in fully bottle fed infants than in fully breastfed infants. Gastroenteritis occurred only in one infant. Breastfeeding was strongly protective against severe infection and also minor infection. Protection continued even after breastfeeding was stopped. Family size,
overcrowding at home, family income and parent’s education did not affect the protection effect of breastfeeding (Ellestad-Sayed et al., 1979).

A study determined the month wise prevalence of feeding patterns. Mothers of 650 infants from 0 to 12 months of age attending a Health Centre were interviewed about current feeding patterns of infants and other socio-economic variables. Breastfeeding was maintained at a high level (more than 90%) throughout infancy but exclusive breastfeeding showed a rapid decline. Less educated mothers with low family income were likely to exclusively breastfeed. 74% of infants were breastfed at 1 month and 48% of infants at 4 months. Between birth and breast feed the time interval was 24-48 hours in 48.9% of the infants. 76.9% of infants received pre-lacteal feeds. Exclusive breastfeeding has to be promoted among pregnant and lactating mothers by health personnel (Chhabra et al., 1998).

The impact of promotion of exclusive breastfeeding on infant health in Guinea-Bissau was evaluated where mortality rates are high. A birth cohort of 1721 infants were randomised for the promotion of exclusive breastfeeding for the first 4-6 months according to WHO recommendation. Water and weaning food introduction was delayed in the intervention group. There was no reduction in mortality in the intervention group when traditional intensive breastfeeding was followed (Jakobsen et al., 2008).

900 mothers were interviewed to identify the reasons for early weaning using a systematic randomised sampling method. 15% of the mothers were illiterate, 93% were housewives, 97% gave birth in hospitals, 68% were bottle fed. 31% of the cases, had not breastfed. 38% used only the milk, whereas 62% used a combination of breast milk and infant formula (Marandi et al., 1993).
52 infants were assessed in an urban community of Lahore in a longitudinal study. 98% of mothers started breastfeeding within the first week and 54.3% continued until 12 months. Pre-lacteal feeds was given to 94% infants. 48% were put on supplemental bottle feeding during first week and 97% were bottle feeding by five months of age. Insufficiency of breast milk was the common reason to start bottle feeding (71%). Illiterate mothers stopped breastfeeding earlier. In the upper socio-economic class and literate mothers weaning occurred earlier. 31% mothers, more in the upper socio-economic and literate group used health professional advice. Exclusive breast feeding should be promoted by health education interventions (Kulsoom et al., 1997).

A longitudinal population based study in UK was conducted to assess breast feeding initiation and rates of exclusive breast feeding for first 6 months after birth and the social class differences in breast feeding rates. 18125 singletons were studied. 71% of babies were breastfed at 1 month, 34% at 4 months, 3% at 6 months. Women in routine jobs were less likely to exclusively breastfeed. In UK, clear social class differences in breastfeeding initiation for first 4 months (Kelly et al., 2005).

650 infants were examined for breastfeeding patterns and role of some factors on exclusive breastfeeding at the Zanjan city, Iran. 92% of infants were breastfed during the first year of life. 82% were exclusively breastfed in the first five days of life, but declined to 44% at first month. Breastfeeding is the major determinants of infant’s health and survival (Koosha et al., 2008).

Current breastfeeding patterns were studied in a retrospective survey in Assam. Breastfeeding is the focus of rapidly growing interest in many areas of demographic research. Early introduction of non-breast milk and early termination of breast milk in the urban society was common (Nath et al., 1997).
The breastfeeding patterns in Central Israel and the association between socio-demographic characteristics in the Tel-Aviv district were studied. 78.5% of mothers initiated breastfeeding. 55.8% infants were breastfed at 2 months, 36.8% at 4 months, 29.9% at 6 months and 11.8% at 12 months, 35.8% of infants at 2 months and 11.2% at 6 months were exclusively breastfed (Berger-Achituv et al., 2005).

The effects of breastfeeding on health services utilisation in 8327 Hong Kong Chinese infants were followed for 18 months in a population based cohort study. Breastfed infants had less doctor visits and fewer illnesses in the first 18 months of life. Infants breastfed exclusively for 2 to 3 months showed strongest results. Compared with exclusively formula fed infants OR for mixed breast and formula fed infants was 2.4; for exclusive breastfeeding 2 to 3 months 3.2 and for exclusive breastfeeding 4 or more months 3.4. Breastfeeding in Hong Kong Chinese infants reduces doctor visits and illness (Leung et al., 2005).

The most important cost effective intervention for protecting children against diarrhoea is breastfeeding. It is effective against all causes of mortality. Nutritional composition and non-nutritive bioactive factors of human milk promotes survival and healthy development of the infant. Increased incidence of diarrhoea and pneumonia is associated with sub-optimal breastfeeding (Turin et al., 2014).

An update in global and regional trends in exclusive breastfeeding from 1995 to 2010 suggests that a common indicator for monitoring and evaluating infant and young child feeding in a given country and region is the proportion of infants 0-5 months who are exclusively breastfed. Prevalence of exclusive breastfeeding among infants younger than six months in developing countries increased from 33% in 1995 to 39% in 2010. Best improvement was seen in West
and Central Africa. Though the well recognised importance of exclusive breastfeeding is wide-spread, Child nutrition programmes worldwide require commitments and investments to improve infant feeding practices to have maximum impact on children's lives (Cai et al., 2012).

Reduced neonatal mortality is associated with initiation of breastfeeding within 1 hour after birth in rural Ghana. In Southern Nepal a community based randomised trial on neonatal mortality and morbidity was conducted. 3.4% of infants were breastfed within 1 hour of birth, 56.6% were breastfed within 24 hours of birth. 72.6% of partially breastfed infants were at a higher mortality risk than exclusively breastfed. 19.1% of all neonatal deaths can be avoided with initiation of breastfeeding within first day. Exclusiveness and duration of breastfeeding should be emphasised to reduce mortality of neonates (Mullany et al., 2008).

Breast milk empties more quickly from stomach than does infant formula. The difference in gastroduodenal motility between neonates fed with human milk and those fed with infant formula was studied. Twenty four 5-36 day old neonates were fed with cow's milk based formula and mother's milk. Post-prandial gastroduodenal contractions were recorded manometrically for three hours. The migrating myoelectric complex, which signals a return to the fasting state, appeared in 75% of breast milk fed infants but only 17% of formula fed infants within the 3 hour recording period (Tomomasa et al.,1987).

The level of efficiency of energy use is determined by the nutrient balance in the diet. Breast milk is a highly complex fluid with a nutrient balance and an array of functional properties. It promotes a level of metabolic efficiency that is not attainable when a cow milk based formula is fed. Field mid clinical studies of
breastfed infant and *in vitro* studies of human milk offer unique opportunities to understand basic mechanism of nutrient intake (Mitchell, 1933).

A study was conducted in Italy to investigate infant feeding practices in the first year of life and factors associated with breastfeeding duration. 91.1% of infants were breastfed. 46.8% and 11.8% of infants were still breastfed at 6 and 12 months respectively. 68.4% and 27.7% received formula, 18.3% and 65.2% were given cow's milk. Negative factors associated with the duration of breastfeeding were lower mother's age, early introduction of formula. Breastfeeding in Italy was still inadequate (Giovannini *et al.*, 2004).

A year long epidemiological study in Cape Town of the epidemiological factors associated with acute infectious infantile diarrhoea was conducted. Breastfeeding was low among coloured population than in black families. Black families originated from a largely migrant population. Coloured infants with diarrhoea came from deprived subgroup of the community. Black infants came from a disadvantaged and impoverished society (Househam *et al.*, 1988).

2383 Norwegian infants aged 6 months were investigated to describe and evaluate infant feeding practices. Breastfed infants population was 96% at 1 month, 65% at 4 months and 80% at 6 months. 1% of the infants were introduced to solid foods before the age of 4 months. Maternal age, education and degree of urbanisation affected exclusive breastfeeding at 4 months, breastfeeding at 6 months. Majority of Norwegian infants are fed according to infant feeding recommendations during their first 6 months of life. Infant feeding practices are associated with infant and maternal characters (Laude *et al.*, 2003).

Based on children who are breastfed according to World Health Organisation recommendations exclusively for first 4-6 months of life a new
international growth reference was prepared. 650 infants were examined in an urban area in Southern Brazil at 1, 3, 6 and 12 months. From 1 to 3 months, partially breastfed infants gained more weight (Victoria et al., 1998).

Breastfeeding prevents infantile gastrointestinal infections and non-gastrointestinal infections. The effects of breastfeeding on health should be done for other reasons and include the collection of related information as part of the study. Breastfeeding is the period of feeding from birth until the infant was weaned. The term bottle feeding referred to no breastfeeding but to an infant formula or cow's milk. Each infant was classified into either breastfeeding group or bottle feeding group. 64% of babies were breastfed at the end of the first month, 43% at the end of the third month and 37.8% at the end of the sixth month. 448 infants with a total of 305 episodes were observed. Gastrointestinal illness was 49% lower in breastfed than in bottle fed infants (Mitosavlevic & Virijevic, 1997).

In a prospective study of Bedouin infants from birth to age 18 months, exclusive breastfeeding was associated with lower rates of infection when compared to older children partially breastfeeding. The benefits of exclusive breastfeeding on the health of infants have been emphasised widely. This study showed lower rates of infection by Cryptosporidium species, lower rates of ear infections and Giardia lamblia infection. In Bedouins and in other populations partial breastfeeding especially at ages 4 to 6 months offers protection against infection, encouraging mothers to continue to at least partially breastfed past age 3 months may help reduce infections and morbidity in infants (Bilenko et al., 2008).

In a case-control study in Brazil showed that infants who are not breastfed are at a 25 time greater risk of dying of diarrhoea than those who are exclusively breastfed. Studies in Peru, India and Nigeria show that breastfeeding should be
continued during diarrhoea. Breastfeeding maintains hydration during diarrhoea. Numerous studies in developing countries prove the need to extend the duration of exclusive breastfeeding for at least 4-6 months. Breastfed infants have a reduced risk of diarrhoeal morbidity when compared to infants who received only water in addition to breast milk. Diarrhoeal disease control programmes need to modify service delivery to ensure that breast feeding mothers are not separated from their infants while on oral rehydration therapy (ORT). Breastfeeding immediately after delivery should be ensured. Appropriate advice should be given on how to breastfeed and counteract breastfeeding problems (Huffman et al., 1990).

120 mother-infant pairs were studied in Shiraz, Iran. 59 mothers had breastfeeding education after delivery and follow up for 4 months. Exclusive breastfeeding rates were higher in the study group (54%). 5% had stopped breastfeeding by age of 4 months. The number of days of diarrhoea in the study group was low. At the end of 4 months, weight and height of the infants was higher in the study group of exclusively breastfed infants. The findings indicated that it is very important to promote breastfeeding and the need for breastfeeding education (Froozanti et al., 1999).

A three year longitudinal study of Bedouin Arab infants in Israel who were breastfed at birth revealed that those born during wet cool months are breastfed longer than those born in dry hot season. 50% were supplemented with milk supplement (Naggan et al., 1991).

Breastfeeding trends were reviewed for the last 30 years. Low income women still lag behind middle and upper income women in stating and maintaining breastfeeding. Breastfeeding efforts of low income women are inhibited by several sources like society, health care profession and the family. Efforts to promote breastfeeding have been initiated at national level.
Breastfeeding promotions include (A) educational efforts to pregnant women and
their families (B) routine and practice change in hospital (C) increasing
breastfeeding support (D) Infant health care education (E) Using media to educate
the public (F) Workplace accommodation for breastfeeding women (G) Continuing
research efforts (Arango, 1984).

The determinants of breastfeeding based on 1113 samples from women in
Alberta, Canada in a National Population Health survey conducted in 1996-97
were assessed. Breastfeeding initiation was 85.6%. 71.3% of mothers continued
breastfeeding for 3 months, 37.2% of mothers breastfed their infants for more
than 6 months. Marital status, education, annual family income and maternal
smoking behaviour were determinants of breastfeeding initiation. Insufficient milk
production during weeks 1 to 12 was the primary reason for weaning (Yang et al., 2004).

483 and 412 individuals were examined at 3 months in the intervention
and control groups. Trained health and nutrition workers counselled mothers for
exclusive breastfeeding: Anthropometry and diarrhoea prevalence at 3 months and
6 months were assessed. Exclusive breastfeeding rates were 79% in the
intervention group. The 7 day diarrhoea prevalence study was lower in the
intervention group. Intervention effect on diarrhoeal morbidity of exclusive
breastfeeding was similar for all births. Exclusive breastfeeding until age of 6
months should be promoted in a developing country through primary health care
services (Bhandari et al., 2003).

A study to investigate practices of breastfeeding in 1039 Sudanese mothers
and to find the influencing factors in urban and rural communities was conducted
in six states. 99.9% of mothers initiated breastfeeding, 83.2% on the first day
between 1-5 hours following delivery. Breastfeeding was 92% at 7 months and
65% at one year. 4% still breastfed at 2 years. Positive attitudes to breastfeeding were still holding. 89.2% stopped breastfeeding due to a new pregnancy and 67.1% stopped if baby had diarrhoea. Despite demographic shifts, shorter duration of breastfeeding was seen in urban affluent mothers in comparison to others (Salih et al., 1993).

In a cross-sectional survey among 235 infant – mother pairs in Enugu, Nigeria in 1998 breastfeeding practices and associated factors were studied. Exclusive breastfeeding rate was 33.3% and predominant breastfeeding rate was 50.2%. Infant’s age, no of breastfeeding counselling attendances and the breastfeeding initiator were compared irrespective of breastfeeding practice. Commonest reasons for not breastfeeding exclusively were insufficient breast milk (58.3%). Health workers should highlight to mothers the dangers of water supplementation and breast milk dynamics for an improvement in exclusive breastfeeding rate through formation of community based lactation support groups (Aghaji, 2002).

A transversal study was performed in Mexico in 220 rural communities with children less than 1 year to determine the prevalence of lactation and factors related to lack of breastfeeding. 21.4% of children were never breastfed in the north, 7.6% in the centre and 5.3% in the South. 35.6% of children were breastfed either exclusively or with mixed feeding. In the second trimester, 67.2% (N), 40.9% (C) and 51.6% (S) had been weaned. Lack of breastfeeding was associated to the health personnel. Early weaning was associated with artificial lactation. In small families artificial lactation and early weaning was common. It is necessary to implement health programs to promote breastfeeding among infant population of Mexican rural communities (Vandale- Toney et al., 1997).
A population based study to determine mortality, prevalence and socio-environmental determinants of diarrhoeal diseases in children less than 5 years of age in the rural area of Akoko North, Ondo state, Nigeria was conducted. 850 children less than 5 years old were selected. 8.1% of the children had diarrhoea. Infant mortality was 102/1000. Social and environmental factors like dirty feeding bottles, poor drinking water storage were related to the high incidence of diarrhoea (Jinadu et al., 1991).

A descriptive survey was done on infants aged between 6 and 12 months of age to determine the relationship between infant feeding practices and diarrhoeal infections. The results of 105 mothers attending the health facilities of Mbabane, Swaziland showed that breastfeeding was routinely practiced by mothers but EBF was very low. Supplementary feeds like formula were introduced within first 3 months of life. Infants on breast milk had fewer diarrhoeal infants. Education and cultural factors influenced feeding mode and diarrhoeal attacks. Education and cultural factors influenced feeding mode and diarrhoeal attacks. Hence breastfeeding should be promoted as an important intervention in control of diarrhoea (Ziyane et al., 1999)

Randomised study of 250 infants for lactation counselling into intervention and control group was conducted. Intervention group mothers were individually advised. All mothers were evaluated for infant feeding practices at home two weeks after discharge. 60% of intervention group mothers were breastfeeding exclusively at discharge compared to only 6 % of control group. 2 weeks later, these rates rose to 75% and 8% in intervention and control groups respectively. 49% reverted back to bottle feeding. Thus counselling had a positive impact to start EBF during hospitalisation. Maternal and health facilities should include lactation counselling as an integral part of their programme to improve infant feeding practice (Haider et al., 1996).
A cross-sectional study was done in a rural and urban area in Tanzania to investigate and compare feeding practices among infants of less than 7 months of age. Exclusive breastfeeding was rarely practiced in either rural or urban areas. Urban mothers started breastfeeding earlier, exclusively breast fed for a longer period, gave breast milk more often and delayed introduction of solids for longer than rural mothers. Urban area mothers needed extended breastfeeding. In both rural and urban areas more efforts are needed to encourage exclusive breastfeeding (Shirima et al., 2001).

A cohort study was conducted in the Islamic Republic of Iran between January 1997 and February 1998 to compare growth and morbidity of 100 infants who were exclusively breastfed for 6 months and 100 who were breastfed and on complementary foods between 4-6 months. Infant's height, weight and feeding pattern were assessed and recorded. The rate of diarrhoea between 4 and 6 months were significantly lower in exclusively breastfed infants (11%) than in complementary food fed infants (27%) and lower respiratory infections (23%) in breastfed and 35% in non-breastfed. Exclusive breastfeeding is superior until an infant is 6 months of age (Khadivzaden et al., 2004).

In a cohort study of children followed for the first 2 years, the relationship between breastfeeding and cognitive development was examined. Children who were breastfed less than or equal to 4 months and those breastfed greater than 4 months were found to be favouring the breastfed children. The direction of the relationship at age of 6 months was the same but did not reach significance (Morrow et al., 1988).

100 mothers of new born babies were studied with regard to their belief and practices about lactation. 98.2% of the mothers were breastfeeding, 87.9% mothers used pre-lacteal feeds of one sort. Only 0.5% breastfed babies within 6
hours and nearly 50% started after 48 hours. 73% wanted to continue breastfeeding beyond 1 year. The age of weaning preferred was after 1 year by 91%. Much attention needs to be taken with regards to proper antenatal care and advice to discourage wrong and harmful feeding practices (Srivasthava et al., 1994).

The energy requirements of infants reflect levels of energy intake that will promote health optimal body composition, levels of physical activity and adequate growth appropriate for their developmental age. Energy density of human milk has been assessed by analysis of its macronutrient composition. This serves as a guideline for the energy content of infant formulas (Annemiek et al., 1994).

2.7 BREAST MILK COMPOSITION

Protein

Mature human milk has the lowest protein concentration among mammals. Based on finding from the WHO study concerning quantity and quality of breast milk, average protein content is 1.15g / 100 ml. The low protein levels in breast milk are more than adequate for optimal growth in young infants and result in an appropriately low solute load for the infant's immature kidneys. True protein content of mother’s milk is about 8-10gm/l (Harzer et al., 1986).

The whey caesin ratio of human milk is 80 : 20 and that of bovine milk is 20 : 80, while that of breast milk substitutes varies from 18 : 82 to 60 : 40. No bovine milk protein is identical to human milk protein. The human whey proteins consist mainly of human alpha lactalbumin, an important component of the enzyme system in lactose synthesis. The dominant bovine whey protein, bovine beta - lactoglobulin, has no human milk protein counterpart. Human milk's high whey : caesin ratio results in the formation of a softer gastric curd, which reduces gastric emptying time and facilitates digestion. Human milk has higher levels of
free amino acids and cystine and lowest levels of methionine, than cow’s milk. Cystine is essential for fetus and pre-term infant. Taurine - another amino acid is high in human milk. Taurine is necessary for conjugation of bile salts (hence fat absorption), in addition to having a role as a neurotransmitter and neuromodulator in the development of the central nervous system. (Raiha et al., 1985).

**Fat**

Human milk provides the breastfed infant with preformed AA and DHA content of about 0.3% of total fatty acids (Koletzko et al., 1992). Docosahexaenoic acid (DHA) and arachidonic acid (AA) constitute a large proportion of the total lipids in the brain and retina (Clandinin et al., 1980). The mean LCP levels of colostrums and preterm milk are higher (Ronneberg & Skara, 1992).

Dietary supply of LCP is necessary for the growth of preterm infant. Preterm infants fed formulas without LCP develop poor AA and DHA, so the preterm infant is unable to elongate and desturate linoleic acid and alpha-linoleic acid to their long chain derivatives (Carlson et al., 1991). The fatty acids in human milk triacylglycerols have a highly specific positional distribution (Martin et al., 1993).

Palmitic acid constitutes 22% of mature human milk lipids and 70-75% of it is esterified at the sn-2 position of the triacylglycerol (Freeman et al., 1965). Palmitic acid in the formulas with vegetable oils is predominantly esterified in the sn-1 and sn-3 position yielding free fatty acids and 2-monoacylglycerol (Bernback et al., 1990).

Cholesterol is synthesised endogenously by the human foetus from the 11\textsuperscript{th} week of gestation and the cholesterol required for brain myelinisation is entirely synthesised within the brain (Carr & Simpson, 1981). Breastfeeding is a natural
method of feeding an infant, the cholesterol level of human milk must be considered physiological (Kallio et al., 1992).

Fat content of mature human milk is ideally suited to the human infant and it evokes a unique physiological response. Fat concentrations increase from 2.0 g/100 ml in colostrum to the mature level of 4 - 4.5 g / 100 ml at 15 days postpartum. Human milk fat is secreted in microscopic globules that are smaller than those in cow's milk.

Triglycerides dominate, with 98% of the lipids enclosed in globules. The fatty acid composition of human milk is relatively stable, consisting of about 42% saturated, about 57% unsaturated, fatty acids. Human milk rich in PUFA is needed for brain development. Breast milk provides 35 - 50% of daily intake in the form of fats. Human milk is uniformly rich in cholesterol.

Carbohydrates

The carbohydrate contribution of human milk for the most part is disaccharide lactose and a rich source of oligosaccharides (complex sugars composed of D-glucose, D- galactose, L- fucose, N- acetylglucosamine and N-acetylneuraminic acid (sialic acid). Acidic or neutral may be classified as the presence or absence of sialic acid. More than 100 oligosaccharides structures have been characterised (Kunz & Rudloff, 1993). The pattern of urinary oligosaccharides in breastfed infants is related to the milk they ingest, explained by intestinal absorption of intact oligosaccharides from human milk (Coppa et al., 1990). Sialylactose is the only complex oligosaccharide present in both human milk and bovine milk (Parkkinen & Finne, 1987).
**Lactose**

Lactose is human milk's major carbohydrate. It is a sugar found only in milk and human milk contains highest concentrations (an average of 4% in colostrum increasing to 7% in mature milk). Lactose furnishes 40% of energy needs. It is metabolised into glucose and galactose needed for development of central nervous system. It facilitates calcium and iron absorption and promotes intestinal colonisation with *Lactobacillus bifidus*. The role of other oligosaccharides in human milk makes up 25% of colostrum and at least one the carbohydrate known as the bifidus factor prevents microbial colonisation.

**Vitamins**

The concentration of beta-Carotene in mother's milk decreases from 2-13 mg/l at day 1 to 0.4 mg/l at day 5 of lactation. Serum level of beta-carotene of breastfed infants increases rapidly whereas it does not rise in formula fed infants. It serves as source of Vitamin A in neonates. It also provides the infant’s defence against oxygen toxicity by quenching singlet oxygen and free radicals (Ostrea *et al.*,1986).

Vitamin concentrations of human milk are almost always adequate for infant needs. Vitamin A is higher in human milk than in cow's milk. Vitamin K is higher is colostrum. After 2 weeks vitamin K supplying intestinal flora will be established in breast fed infants. Vitamin E content is adequate. Vitamin D content of human milk is low (0.15 mg / 100 ml). Vitamin $B_{12}$ is very low but its bioavailability is enhanced by a specific transfer factor.
**Minerals**

Human milk is low in calcium (200-350mg/l) and phosphorus (110-160mg/l) (Gross *et al.*, 1980). Though the calcium and phosphorus levels are higher in infant formulas, bone mineralisation is same in breastfed and formula fed infants (Hillman *et al.*, 1988).

Low phosphorus content of human milk is advantageous to the infant, due to the immature renal handling of phosphates in the newborn. Serum phosphorus levels are increased when phosphorus intake is high (Manz, 1992). High serum phosphorus levels have clinical consequences; hypocalcaemic tetany may occur in healthy term infants receiving a high phosphorus formula (Venkataraman *et al.*, 1985).

**Iron**

The very low content (0.5 mg/l) of human milk exhausts its iron stores until four months of age. This is attributed to the high absorption of iron from breastmilk (Saarinen *et al.*, 1977).

High Selenium intake of breastfed infants as well as higher availability of human milk selenium are seen in higher serum selenium concentrations and a higher selenium retention compared to formula fed infants (Kumpulainen *et al.*, 1987).

Chromium content of infant formulas (5-25ug/l) is higher than that of mother’s milk (0.2-0.5ug/l) (Deelstra *et al.*, 1988).

Molybdenum levels in mother’s milk is 1-3ug/l (Casey & Neville *et al.*, 1987).
The concentrations of most minerals in breast milk (e.g.) calcium, iron, phosphorus, magnesium, zinc, potassium and fluoride will not be significantly affected by maternal diet. Mineral concentrations are lower in human milk than in any breast milk substitute and thus better adapted to the infant's nutritional requirements and metabolic capacities. Calcium is more efficiently absorbed because of human milk's high calcium : phosphorus ratio (2:1). The high bioavailability of human milk iron is the result of a series of complex interactions between the components of breast milk and the infant's body. Zinc and copper are found in appropriate levels. The transfer factor Lactoferrin, which prevents iron from being available to intestinal bacteria and releases it only when specific receptors unlock the lactoferrin molecule. All these factors are important to increase iron absorption. Upto 70% of breast milk iron is absorbed when compared to 30% in cow's milk and 10% in breast milk substitutes.

**Trace elements**

Copper, Cobalt, Selenium, levels in human milk are higher than in cow's milk. At three months of age, selenium status is better in exclusively breast fed infants than in artificially fed infants. Iodine is concentrated in human milk.

Inositol is present in mature mother’s milk in an amount of 250-300mg/l, which is a component of membrane phospholipids and of compounds involved in signal transduction. Formula milks are low in inositol, serum inositol levels are higher in breastfed infants (Bromberger & Hallman, 1986).

Taurine effects on cholesterol synthesis, bile acid secretion, fat and vitamin D absorption and auditory brainstem evoked responses have been shown in preterm infants (Tyson *et al.*, 1989).


**Nucleotides**

Breast milk contains significant amount of nucleotides. Dietary nucleotides in infant nutrition has been studied extensively in the last decade. Clinical evidence found that nucleotides enhanced the growth of bifidobacteria in the faecal flora of infants (Gil et al., 1986). Dietary nucleotides increased indices of cell mediated immunity in infants without influencing the incidence and severity of infections (Carver et al., 1991). Infants living in contaminated environment fed a nucleotide supplemented formula, experienced less diarrhoea (Brunser et al., 1994).

**Enzymes**

The dozens of enzymes in human milk have a multifunctional origin. Some reflect the infant's digestive enzymes - alpha amylase and bile salt stimulated lipase. Proteolytic enzymes, peroxidases, lysozyme and Xanthine oxidase are important for neonatal development. Lysozyme concentration is 5000 times greater than cow's milk. This enzyme is bacteriolytic against gram positive bacteria and some viruses.

Human milk is uniquely superior for infant feeding and is species-specific, all substitute feeding options differ markedly from it. The breast fed infant is the reference or normative model against which all alternative feeding methods must be measured with regard to growth, health and development.

Epidemiologic research shows that human milk and breast feeding of infants provide advantage with regard to general health, growth and development.

Research in the United States, Canada, Europe and other developed countries, among predominantly middle class population, provides strong evidence
that human milk feeding decreases the incidence or severity of diarrhoea (Dewey 
et al., 1990, Kovar et al., 1984, Popkin et al., 1990, Beaudary et al., 1995).

A few factors in milk like anti-antibodies (anti-idiotypic antibodies) and T
and B lymphocytes in some experimental models have been able to transfer
priming of the breastfed offspring. This together with transfer of numerous
cytokines and growth factors into milk may add to an active stimulation of the
infant's immune system.

Breast-feeding has an unique capacity to stimulate the immune system of
the offspring possibly with several long term positive effects. Breast-feeding
provides passive and long-lasting active Immunity (Hanson et al., 1998).

The role of breast milk in the intestinal colonisation of the preterm infant
was studied in 23 low birth weight infants and in the control of epidemic diarrhoea
due to Enteropathogenic E.coli in nurseries. 12 of the cases were breastfed and
eleven were bottle fed with cow's milk. Bacteriologic studies of the faeces daily
for first 18 days of life revealed Staphylococcus aureus in 5 cases given breast
milk and 8 receiving cow's milk. EPEC was found in 3 cases with breast milk and
in 6 with cow's milk. Proteus was isolated from 10 cases with breast milk and 3
cases with cow's milk. None of the cases fed breastmilk had diarrhoea including 3
cases with E.coli. In another group, 4 cases developed severe acute diarrhoea, due
to enteropathogenic E.coli followed by 2 deaths. The study results point out that
although colonisation by enteropathogenic strains of E.coli, these strains do not
cause disease in breastfed infants (Fernandez et al.,1979).

A cohort study conducted in Chittagong, Bangladesh revealed the
association between infant feeding pattern and diarrhoeal illness where exclusive
breast fed infants showed low prevalence of diarrhoea than bottle fed infants (Seema et al., 2008).

Exclusive breast feeding to 6 months of age is recommended because it protects against infections morbidity, mortality and promotes adequate growth and development (Karma et al., 2001).

93 Mexican breastfeeding infants stated that the pattern of oligosaccharides varied among milk samples; in each milk sample, the pattern was summarised as a ratio of 2-linked to non 2 linked fucosyl oligosaccharides. Milk with higher 2 linked fucosyl oligosaccharides ratios affords greater protection against infant diarrhoea. Specific oligosaccharides constitute a major element of an innate immune system of human milk (Newberg et al., 2004).

52 mothers in rural South Eastern Kentucky were examined for infant feeding practices at 1-2 months of age. 52% used formula milk at birth, 41.2% practiced breastfeeding and 8% were both breastfeeding and formula feeding. By end of 1 month only 29% were breastfeeding. At 4-6 months post-partum 80% of mothers were formula feeding and 20% were breastfeeding. Mothers with more children, more education and higher family income breastfed longer. Breastfeeding support was required to continue breastfeeding beyond the first month. Education to solid food and formula to infants should be implemented (Barton et al., 2001).

73 breastfed infants and 65 formula fed infants were compared for their growth pattern in Italy. Breastfed groups showed better growth from 0-6 months (Agostoni et al., 1999).

Infant feeding malpractices associated with mother's low level of education are lack of breastfeeding and early introduction of cow's milk. 400 mothers of
infants below 12 months of age were divided into 3 groups. Type of milk given at birth, 1, 3 and 6 months of age and data analysis showed that infants born to mothers of low and intermediate education did not receive exclusive breastfeeding compared to those with higher level of education. Prevalence of exclusive breastfeeding was 37%, 40% and 65% in infants aged 3 months in the three groups. In 6 months old infants, prevalence rates were 13%, 15%, and 48%. Cow's milk was given at 3 months of age old infants belonging to mothers with low level of education compared to those with intermediate education (12% vs 5%). At 6 months of age with prevalence rates for cow's milk feeding of 39%, 20% and 0% in the three groups. Data support low education level and infant feeding malpractice. Paediatricians should play an effective role to support adequate duration between breastfeeding, formula and cow's milk (Ummarino et al., 2003).

In a US based study of sample size 2907 at birth to 1782 at 12 months of age, rapid transition of an infant diet from milk to varied diets takes pace. 83% of the survey respondents started breastfeeding which declined to 50% at 6 months to 24% at 12 months. 52% infants received formula. 40% consumed cereal at 4 months. Breast milk was supplemented with infant formula while infants were still in hospital. In spite of recommendations that supplements should not be introduced to infants 4 months or younger, almost half the infants consumed solid foods by the age of 4 months. Early complementary food introduction was associated with unhealthy feeding habits (Grummer-Trawn et al., 2008).

Odds of breastfeeding beyond 4 weeks or to the recommended 6 months (Exclusive breastfeeding) was significantly less than term infants. Late preterm infants are at risk for breastfeeding associated rehospitalisation and poor breastfeeding establishment compared to pre-term infants (Radtke, 2011).
The microflora and pH of gastric contents in breast fed and bottle fed infants with acute diarrhoea were determined which showed that bottle fed infants had low pH values and low bacterial concentrations whereas infants with chronic diarrhoea had high pH rates and bacterial overgrowth, essentially of Gram-negative bacilli. Breast-fed normal infants had Hydrogen-ion concentrations similar to those of the chronic diarrhoea group, but without Gram negative bacilli overgrowth (Maffei et al., 1995).

In a prospective community based study in West Bengal, exclusively breast fed infants for 4 months did not suffer from diarrhoea when compared to artificially fed infants. The incidence rate ratio was 1.02 (95%) (Mondal et al., 1985).

No significant differences were found in the frequency of dysentry between breastfed and bottle fed infants in a study conducted in Nigeria. Persistant diarrhoea was less common in breast feeding children than artificially fed infants (Meremikwu et al., 1997).

Among poor rural infants, artificial feeding was associated with a four fold difference in diarrhoea rate between exclusively breast fed and bottle fed infants in the first 4 months of life (Kumar et al., 1981).

In a study conducted among rural infants 0-2 month, early weaning was associated with the risk of diarrhoea (Mondal et al., 1990) Escherichia coli was isolated from 17% of children and 40% of mother's hands, 59% of leftover drink and 30% of utensil's swabs. Lactoferrin content in breast milk is high during first 3 months after child birth protects children from diarrhoea in early infancy.
2.8 NUTRITIONAL REQUIREMENTS

Energy needs

The total daily calorie intake of infants is utilised in the following ways.

1. About 50% supplies basal metabolic requirements.
2. 12% is needed for tissue growth.
3. 5% is involved in the specific dynamic effect of food ingestion, especially, protein, in general and for stimulation of metabolism.
4. Various physical activities require 25%.
5. 8% is represented on faecal loss.

<table>
<thead>
<tr>
<th>Age</th>
<th>Kilocalories / Kg (Kcal / Kg)</th>
<th>Kilojoules / Kg (Kj / Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upto 3 months</td>
<td>120</td>
<td>500</td>
</tr>
<tr>
<td>3 - 5 months</td>
<td>115</td>
<td>480</td>
</tr>
<tr>
<td>6 - 8 months</td>
<td>110</td>
<td>460</td>
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</table>

A few factors in milk like anti-antibodies (anti-idiotypic antibodies) and T and B lymphocytes have in some experimental modes been able to transfer priming of the breastfed offspring. This together with transfer of numerous cytokines and growth factors via milk may add to an active stimulation of the infant's immune system (Hansen, 1998).
In a study of 10,947 Breastfed singleton infants born in rural Ghana between July 2003 and June 2004 the risk of neonatal death was fourfold higher in children given milk based fluids or solids in addition to breast milk. There was a marked dose response of increasing risk of neonatal mortality with increasing delay in initiation of breastfeeding from 1 hour to day7; overall late initiation (after day1) was associated with a 2.4 fold increase in risk. Promotion of early initiation of breastfeeding has the potential to make a major contribution to the achievement of child survival goal. 16% of neonatal deaths could be saved if all infants were breastfed from day 1 and 72% if breastfeeding started within the first hour (Edmond et al., 1999).

20 breastfeeding infants aged 2 days to six days were measured for breast milk consumption. Infants were tested before and after every feeding over a 24 hour period. Significant correlations were found between the number of feedings per day and breast milk consumption when examining all twenty infants and ten partial breast feeders but not the ten full breast feeders (Novotny et al., 1983).

Attributable fractions for death due to diarrhoeal disease are 55% for the first 6 months of infancy. Globally as many as 1.45 million lives are lost due to suboptimal breast feeding in developing countries (Lauer et al., 2006).

The association between breastfeeding dose and illnesses in the first 6 months of life was examined. Most breastfeeding infants had lower odds ratios for diarrhoea compared with no breastfeeding (Raister et al., 1999).

In a birth cohort study throughout Bavaria, Germany 474 infants were exclusively breastfed for > or = 6 months (group A), 870 infants were exclusively breastfed for or = 4 months (group B) and 619 infants not breastfed for 4 months (group C). In multivariate analysis OR = 6 months of exclusive breastfeeding
reduced significantly the risk for OR = 1 episode of gastrointestinal infection compared to, 4 months breastfeeding. The findings supported the recommendation for 6 months of exclusive breastfeeding (Rebhan et al., 1998).

In a study of babies weighing greater than 2.5 kg and 126 babies weighing less than or equal to 2.5 kg at birth were studied. The study was done for 6 months. 273 were breastfed babies and 234 artificially fed babies. Artificially fed had a mortality rate of 21.6 while breastfed had a low rate of 15.8. For 1-6 months period a mortality rate per 1000 live births of 53.8% was found for term babies breastfed at the rate of 29.9%. LBW had a rate of 44.9 while those weighing more than 2.5 kg at birth, a rate of 9. Mortality rate for term babies in early infancy can be reduced by promotion of breastfeeding (Aswathi et al., 2002).

Researchers are delineating many of the unique anti-infective and nutritional factors in human milk. A mother who breast feeds makes a significant contribution to her infant's health. Contraindications to breastfeeding are few and relate primarily to medications and maternal illness. Health personnel and physicians should advice the mother on breastfeeding so that breastfeeding can be continued into the first year of the infant's life (Oseid, 1979).

In developing communities, exclusive breast feeding is encouraged since it has been found to be protective against infantile diarrhoea. A study in Qatar explored the relationship between breastfeeding and diarrhoea and to assess the effect of Exclusively Breast Fed on the risk reduction of diarrhoea in children aged 0-5 years. The socio-demographic characteristics, feeding modes and diarrhoeal morbidity were collected from parents. When compared to the Exclusively Breast Fed infants, the risk of diarrhoea was higher and statistically significant in both the partially breast fed and in the Non-Exclusively Breast Fed (Mohammad et al., 1998).
In a cohort study of 133 low birth weight infants and 260 appropriate birth weight were followed for the first 6 months of life. All were from poor families living in the interior of Pernambuco, North East Brazil. None had congenital abnormalities. Infant deaths, hospitalisation and morbidity data were collected prospectively through daily home visits from birth through week 8, then twice weekly for weeks 9 to 26. Of the Low Birth Weight infants, 56% were wasted, 23% stunted and 17% both stunted and wasted. Low Birth Weight infants experienced a seven fold higher mortality rate and four fold higher rate of hospitalisation (Lira et al., 1996).

The feeding patterns of Chilean children up to 18 months old and their relation to nutritional status were studied. 102 of the 320 urban health clinics located throughout the country were selected. The interview helped to identify the type of feeding (exclusive breastfeeding, exclusive bottle feeding, breast feeding plus bottle feeding). The results demonstrated the benefits of exclusive breastfeeding through the first 6 months of life, the need for exclusive breast feeding with solid food after that time, and the superior nutritional status of breast fed children within the age groups studied (Castillo et al., 1996).

The increased risk of diarrhoeal infections and general poor quality of the supplementary foods mean that supplementary feeding before the age of 6 months is unlikely to lead to a growth advantage and may lead to growth faltering (Rogers et al., 1997).

Breastfeeding was declined worldwide since 1930. Breastfeeding practices between developed and developing countries and between rural and urban people are different. Anthropometric measurements and dietary data collections were done mostly for the first one year. 100% rural, 98% urban poor, 78% urban elite mothers breast fed their babies at birth. At 1 year 97% rural, 90% urban poor and
25% elite continued breastfeeding. Only some rural children but many of the urban children were provided with cow’s milk during breastfeeding (Khan, 1980).

The literature on the relationship between infant feeding mode and the relative risk of diarrhoeal morbidity in 0-3 months, 3-5 months, 6-8 months, 9-11 months and 12-23 months was reviewed. A dose response relationship between relative risk of diarrhoeal morbidity and infant feeding mode was found, with bottle-fed infants having the highest risk, partially breastfed an intermediate risk and the exclusively breastfed infants having the lowest risk. The association between infant feeding mode and diarrhoeal risk is also related to infant age. The relative risk is 3 for infants aged 0-3 months for exclusively breastfed versus non-breastfed infants and 2.4 for infants aged 3-5 months. No association was found between risk of diarrhoeal disease and breastfeeding once breastfeeding had ceased, emphasising the protective effect of breastfeeding lasted only while breastfeeding continued (Feachem et al., 1984).

The effect of feeding on infant morbidity and mortality was studied in urban Philippines in a cross-sectional study of 138 infants with diarrhoea. 90% were formula fed, 6% were partially breastfed and 4% were exclusively breastfed. Mode of infant feeding was related to mortality. 67 infants died of which 96% were formula fed, 1% were partially breastfed and 3% were exclusively breastfed. Rooming-in and introduction of formal breastfeeding policies, increased the proportion of infants exclusively breastfed by 135% and the death incidence of clinically infected new-borns dropped by 95.3% (Clavano et al., 1982).

Breastfeeding practices were studied over the first 6 months of life among urban poor infants in Southern Brazil. The duration of breastfeeding was associated with the following: the infant's sex, mother, type of first feed, timing of the first breastfed, breastfeeding regimen and frequency of breastfeeding at 1
month. The following were significant risk factors for early termination of breastfeeding: the infant's sex, type of first feed, use of supplementary feeds, frequency of breastfeeding, feeding regimen, weight for age and weight for age after controlling for birth weight. Dissatisfaction was the most frequent reason given by mothers (Martines, 1989).

The determinants and incidence rate of bottle feeding during the first month of life was assessed and its effect on breastfeeding was noted. 211 infants in the city of Porto Allegre, Southern Brazil were examined. By Day 7, 21.3% of infants were bottle fed and 46.9% were bottle fed by Day 30. Factors associated with bottle feeding at Day 3 were pacifier use at Day 7 and nipple trauma at Day 7. At Day 30, the breastfeeding technique was more adequate among exclusively breastfed infants than those who were also bottle fed. The results showed that teenage mothers started bottle feeding in the first month. Bottle feeding negatively affects breastfeeding technique (Franca et al., 2008).

480 Jewish infants visiting pediatric emergency room (ER) with infectious diseases were compared for breastfeeding habits with 500 healthy infants. Breastfeeding was less prevalent among ER infants than healthy infants. 22.6% had gastroenteritis. A very short breastfeeding period of 2 weeks or less was more prevalent among ER group and increased hospitalization rate. The data emphasises the importance of breast milk in reducing ER visits and hospitalisation rate (Dagan et al., 1982).

Infant feeding practices were studied in Western Australia and Tasmania in 1984-1985. In Western Australia and Tasmania, 86% and 81% respectively, were breastfeeding their babies on hospital discharge. 45% of all mothers were still breastfeeding and the length of lactation had an significant effect on the social rank of the family. Higher social group mothers breastfed their infants for longer
than did mothers of lower social groups. Solid foods were introduced for few infants before three months of age, solid and non milk foods were introduced earlier to infants who were fed artificially than those who were breastfed (Hitchkock *et al.*, 1988).

39 infants were studied for nutrient intakes by a combination dietary record at 2, 4, 8, and 12 weeks of age. Infants were grouped according to feeding method: human milk only, formula only, formula plus solid foods and human milk plus solid foods. The effect of feeding regimen upon renal concentrating capacity was assessed by determining plasma osmolality, sodium and urea in blood samples drawn at 4 and 8 weeks of age. Results indicate that milk was the major source of energy and of ten of the thirteen nutrients tabulated at all ages examined. Data also suggest that solid foods replace, rather than supplement, human milk or formula on a caloric basis. There was significant differences in dietary renal solute observed between breastfed and formula fed infants (Matin, 1980).

The epidemiology of early weaning would help prevent infant mortality and birth rates in developing countries from rising where the bottle feeding practice can be expected to increase with economic progress. Village and urban mothers in Chile wean their babies early in Chile which may be responsible for Chile's high infant mortality relative to its economic and social levels. Infant feeding practices were investigated to clear the respective roles of maternal milk deprivation and factors associated with bottle feeding and to measure the effect of early weaning on mortality.

Breast feeding was less common now than it had been 15 or 20 years ago. The decrease in breast feeding in the study area was ascribed to use of bottles by mothers. More than two thirds feel that breast feeding should be continued for at least a year (Plank *et al.*, 1993).
The optimal duration of exclusive breastfeeding and the health benefits and the effects on child health, growth and development of exclusive breastfeeding for 6 months vs exclusive breastfeeding for 3-4 months followed by mixed breastfeeding to 6 months was systematically reviewed. 20 independent studies meeting the selection criteria were identified from 2 developing and 11 developed countries. Infants who were exclusively breastfed for 6 months showed good weight gain. In Belarus, infants who continue breastfeeding for 6 months or more appear to have a reduced risk of one or more episodes of gastrointestinal infection. Infants on breastfeeding exclusively for 6 months experience less morbidity from gastrointestinal tract infections (Kramer et al., 2004).

Infant feeding practices in rural area of Bangladesh was done as a Longitudinal study. 110 infants were followed up from birth to 1 year of age by alternate day home visits. 100% of mothers breastfed their infants from birth to 1 year. Bottles containing various kinds of milk and starchy food were added to 60% of infant diets by 3 months and 80% by 5 months of age. Additional food was tinned milk. Infant feeding practices in their population is improper and mothers should be trained and motivated on weaning practices (Das et al., 1992).

52 mothers and infants in a rural area of Northern Thailand were studied for 48 hours. Mean 24 hour breast milk intake was 579 gm. The proportion of 24 hour breast milk intake consumed at night ranged from 8 to 91%. Positive associations were found between breast milk intake, sucking time and infant weight (Imong et al., 1990).

The nutritional and protective characteristics of breastfeeding as a biological need on infant feeding, it is still the best system of feeding in spite of advances in the composition of formula milks. In a study in Spain, at birth 73% of the mothers began to nurse their babies, 19.2% used formula and 7.3% used other
feeding methods. 90.7% had positive opinions about breastfeeding. Supplementation of breast milk is established early since 1 month of age. 32.1% of infants received mixed nutrition (Moran Rey, 1992).

In a study of 1677 infants born in slum areas of Dhaka, Bangladesh it was observed that the portion of infants who were exclusively breastfed was only 6% of enrolment, increasing to 53% at 1 month and then gradually declining to 5% at 6 months of age. Predominant breastfeeding declined from 66% at enrolment to 4% at 12 months of age. Proportion of partially breastfed infants increased with age. Very few infants were not breastfed. Breastfeeding practices did not differ between low and normal birth weight infants at any age. The overall infant mortality rate was 114 deaths per 1000 live births. Compared with exclusive breastfeeding in the first few months of life, partial or no breastfeeding was associated with 23 fold higher risk of infant deaths resulting from all causes and 2.40- and 3.94-fold higher risk of deaths attributable to ARI and diarrhoea respectively. The important role of appropriate breastfeeding practices in the survival of infants is clear from this analysis. The broad based beneficial effect of exclusive breastfeeding in prevention of infectious diseases and its role in reducing exposure to contaminated food, which may have contributed to the strong protection against diarrhoeal deaths (Arifeen et al., 2001).

In a cohort study in Netherlands, rates of breastfeeding during the first 6 months (never, partial for < 4 months, not thereafter, partial for 4-6 months, exclusive for 4 months, not thereafter, exclusive for 4 months, partial thereafter and exclusive for 6 months) were assessed. Compared to never breastfed infants, those who were breastfed exclusively until the age of 4 months and partially thereafter, had low risk of GI infections. Similar tendencies were observed for infants who were exclusively breastfed for 6 months or longer. Partial breastfeeding even for 6 months, did not result in significantly lower risks of
these infections. Exclusive breastfeeding until the age of 4 months and partially thereafter caused reduction of gastrointestinal morbidity in infants. Exclusive breastfeeding for at least 4 months but preferably 6 months was advised in industrialised countries (Duijits et al., 2010).

Protective effects of breast milk may derive from an altered mucosal colonisation pattern in breastfed infant. Human milk contains anti-infectious potential such as IgA, oligosaccharides and glycoproteins and cytokines which are associated with protection from gastroenteritis, upper and lower respiratory tract infections, acute otitis media, UTI, neonatal septicaemia and necrotising enterocolitis. Breastfed infants may be colonised by diarrhoeal pathogens and still be healthy. Secretory Ig A in breast milk protects from translocation of intestinal bacteria across the gut mucosa by coating intestinal bacteria and blocking their interaction with the epithelium. Breast milk is also highly anti-inflammatory and contain hormone like factors which counteract diarrhoea (Wold et al., 2001).

The lack of exclusive breastfeeding among infants 0-5 months of age and no breastfeeding among children 6-23 months of age are associated with increased diarrhoea morbidity and mortality in developed countries was reviewed. They estimated the protective effects conferred by varying levels of breastfeeding exposure against diarrhoea incidence, diarrhoea prevalence, diarrhoea illness. Eighteen included studies indicated varying degrees of protections across levels of breast exposure conferred by exclusive breastfeeding among infants 0-5 months of age and by any breastfeeding among infants. Not breastfeeding resulted in excess risk of diarrhoea mortality feeding exposure with greatest in comparison to exclusive breastfeeding among infants 0-5 months of age. WHO recommendation for exclusive breastfeeding during the first 6 months of life is a key child survival tool. These findings highlight the importance of breastfeeding to protect against
diarrhoea-specific morbidity and mortality throughout the first 2 years of life (Lamberti et al., 2011).

The immune response in breastfed and bottle fed children with diarrhoea at Paediatric division observation ward, Kenyatta National hospital and Maternal and child health clinic, Punwani maternity hospital was compared. Stools of 457 children were cultured for bacteria. 69.6% of whom presented with diarrhoea. Breastfed children had a shorter duration of diarrhoea than either mixed fed or bottle fed. *E.coli* was less commonly isolated from breastfed than mixed fed or bottle fed. *E.coli* was more frequently isolated from bottle fed children who presented with diarrhoea than without (Kakai et al., 1995).

In a multistage, randomly selected samples of 1039 Sudanese mothers, infant feeding and weaning practices were investigated. 77.9% believed that breast milk was best for their babies by 6 months. 82.5% in urban middle and high classes started food supplementation compared to urban poor class and the rural group. Weaning started between 6 and 12 months. 27% and thereafter in 64.9%, 36.5% of rural mothers weaned their babies after the age of 18 months the first food item of choice for weaning was fresh goat's or cow's milk (77.6%) followed by powdered or formula milk (16.1%) (el Bushra et al., 1994).

A study of breastfed and bottle fed infants in the first days of life reported that, breastfed infants consume minimal amounts of milk which may be explained by limited milk output or self limitation. The spontaneous formula intake of unrestricted formula fed infants to that of breastfed infants over the first 48 hours of life were compared and hypothesised that spontaneous formula intake of unrestricted infants is much higher than that of breastfed infants and spontaneous formula intake correlates with gestational age or birth weight. New born infants offered formula milk every four hours consumed much larger amounts than
breastfed infants. Weight loss was more marked in breastfed infants on day 2 of life. (Dollberg et al., 2001).

The health advantages of breastfeeding for infants in rural and urban areas of United States and other industrialised nations were studied. The association between infant feeding mode and a large number of outcome measures were established. Breastmilk is associated with small but consistent differences in cognitive tests, diarrhoeal disease, lower respiratory disease and otitis media (Heineg et al.,1996).

In a prospective community based study the relationship between diarrhoea and infant feeding patterns in a rural community in West Bengal was examined. Results showed that the duration of exclusive breastfeeding is short. 2 groups of infants were: breastfed exclusively for 4 months (weaned late) and those infants who were not exclusively breastfed (weaned early). Early complementary feeding was associated with 3 times the risk of diarrhoea (Mondal et al.,1996).

The influence of breastfeeding on the occurrence of dysentery, persistent diarrhoea among Nigerian children with diarrhoea was studied. No differences in the frequency of dysentery was seen between breastfed and non-breastfed infants. Persistent diarrhoea was significantly less common in breastfeeding children than in those who had stopped breastfeeding (Meremikwu et al.,1997).

In a longitudinal analysis of infant morbidity and extent of breastfeeding in the United States established a dose-response association between breastfeeding and diarrhoea. The infants were grouped as (1) breastmilk (100% breastmilk) only (2) mixed feeding (breastmilk and formula milk), (3) Formula milk only (0% breastmilk). Mixed feeding was separated into high, middle and low mixed feeding, representing 89-99%, 58-88% and 1-57% respectively, of feedings of
breastmilk. Formula fed infants showed an 80% increase in the risk of developing diarrhoea (Scariati et al., 1997).

Breastfeeding was adequate in the rural area of Malawi. 46 infants were studied for 10 months. Prevalence of infectious diseases gradually increased. Highly contaminated water was given to babies from first day of life. In spite of this, no diarrhoeal episodes were seen during first 5 months but respiratory tract infections and fever episodes were common. Diarrhoea was a problem from 5-6 months (Lindskog et al., 1994).

In two paediatric cohorts in a low socio-economic level community in Santiago, Chile, determined the incidence of diarrhoea due to six categories of diarrhoeagenic Escherichia coli 340 children were examined. 10-12 newborns were examined for 12 months. Diarrhoeal episodes were detected by twice weekly household visits. E.coli from stool cultures were hybridised with DNA probes specific for enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, enteroaggregative and diffuse adherence E.coli. Incidence of diarrhoea was low. Enteropathogenic E.coli was found in large proportion of diarrhoeal episodes and were incriminated during first year of life. Enteroinvasive and enterohemorrhagic E.coli were isolated at a low rate (Levine et al., 1993).

In a cohort study of 75 rural infants suffering from diarrhoea followed longitudinally during the first year of life between 1985-1987 in the village of Lugar Sobre la Tierra Blanca, Morelos state, incidence of colonisation by enteropathogenic, enterotoxigenic, enteroinvasive and enterohemorrhagic Escherichia coli (detected by DNA hybridisation and specific radio labelled probes), Salmonella species, Shigella species were related. 82% of the children suffered from diarrhoea.64% of the children had enteropathogenic E. coli or Shigella species. 25% was the isolation rate for enterotoxigenic E. coli, Salmonella
species. Enteropathogenic *E. coli* adherence factor, human heat stable enterotoxins, fimbrial colonisation factor antigens and Shiga like toxins I and II were the pathogenic characteristics (Craviota *et al.*,1990).

### 2.9 BOTTLE - FEEDING

Diet, together with other environmental factors, plays an important role in health and disease. Enhancing infant formulae to mimic the health promoting benefits of breast milk has been of significant interest in recent years. Differences in the gut microflora of breast fed and formula fed infants have been reported, which may explain disparities in infection rates between these two groups. Various functional foods such as probiotics, oligosaccharides and proteins, when added to infant formulae could beneficially modify the composition of the gut microflora in formulated infants (Kain *et al.*, 1989).

The bacterial contamination of the lacteal contents of feeding bottles prepared in urban low and high socio-economic groups in Sao-Paulo, Brazil was evaluated. *E.coli* was isolated from 26% of samples from low urban group and 6% from high urban groups. In the high urban group, higher coliform counts were associated with food handlers other than mother. Feeding bottles using tap water were heavily contaminated in the low urban group (Morais *et al.*,1998).

Microbial contamination of 75 uncleaned and 150 cleaned feeding bottles in South wales, UK was evaluated. Microbial contamination of powdered infant formula (PIF) is known to cause gastrointestinal infections in infants. Enterobacteriaceae and *Staphylococcus aureus* were isolated from 12-15% of unclean bottles and contamination was detected from screw caps and teat interiors. In cleaned bottles 4% had *Staphylococcus aureus*. Effective cleaning of bottles should be implemented (Redmond *et al.*,2009).
500 infants under 6 months of age using bottle milk were selected and the bacterial contamination of the bottle milk were examined in Bangkok. 91.8% (459/500) bottle milk samples had bacterial contamination. 82.8% (380/459) had enteric bacteria. 56.6% of Klebsiella species, 41.3% Enterobacter species, 13.4% E. coli, 1.8% Vibrio cholera non O-1 were isolated. 7.8% (4/51) of the E. coli were identified as Enteropathogenic E. coli (EPEC). 74% of the contaminated bottle milk contained one type of bacteria, 23.7% had two types and 2.3% had 3 or more types of bacteria. Promotion of breastfeeding should be encouraged. Hygienic preparation of bottle milk and feeding practice should be emphasised (Suthienkal et al., 1999).

Samples of water from incubator system, health care worker hands and feeding bottle preparation room of a neonatal intensive care unit were collected. Pseudomonas aeruginosa was isolated from 30 neonates from September 2004 to December 2004. 9 out of 48 samples from the feeding bottle preparation room were positive for Pseudomonas aeruginosa. Discontinuation of in-house preparation of feeding bottles stopped the outbreak. Solution of milk from multidose powder preparation may be the source of P. aeruginosa infection (Sanchez-Carillo et al., 2009).

The microbiological quality of pasteurised milk was evaluated in Rio de Janeiro, Brazil and serologically determined enteropathogenic Escherichia coli (EPEC) strains from milk samples. 90 samples of 3 different commercial brands were tested. Total and coliform bacteria were estimated using MPN technique. 208 strains of E. coli were isolated of which 46 (22.1%) were serologically classified as EPEC. Isolation of EPEC serogroups from pasteurised milk represent a risk for children (Da silva et al., 2001).
UK surveys have found that reconstituted bottles are often not stores in a refrigerator but left on the kitchen work surface or in the bedroom. In one survey in Birmingham, 10% of mothers failed to follow manufacturer's instructions and added powder before water when making up the bottles, 20% added an extra scoop of powder to help their infants sleep and 30% of mothers used microwave ovens to heat up milk. There is considerable potential for harm when formula products are not reconstituted or stores safety. Improper sterilisation of equipment or storage of reconstituted feed could lead to contamination by micro-organisms and this could cause gastrointestinal irritation and diarrhoea. Studies of bottles and teats said to have been cleaned adequately have shown unacceptable bacterial counts in over half the samples. In a study, mothers who kept prepared formula at room temperature for more than 2 hours reported higher rates of diarrhoea in their infants. It has been demonstrated that bottle feeding is higher in women who are younger and less educated (FeIn and Falci, 1999).

In preterm infants, the levels of AA and DHA in plasma and erythrocyte phospholipids of term, formula fed infants are lower than of breastfed infants (Clarke et al. 1992). When the linoleic acid : alpha-linoleic acid ratio of a term formula is decreased to 4:1 improves the DHA status of the infant (Clark et al., 1992).

The British Nutrition Foundation (1992) recommends AA and DHA amounts in infant formulas similar to those of human milk and acknowledges the addition of LCP for preterm formulas.

A minimum daily of 0.2 g DHA/100gm fatty acids should be incorporated in formulas designed for preterm infants to prevent cerebrocortical deficiency of DHA (Farquharson et al., 1992; 1993).
Infant formulas have been developed in the last decades closely approach human milk in nutrient composition. Different substances have been incorporated into infant formulas to serve nutritional roles and mimic breast milk. In the near future infant formulas will be fortified with selenium to levels found in mother’s milk (Kumpulainen et al., 1987).

No formula can supplant mother’s milk as the ideal food for healthy term infants. Biological properties of human milk make it uniquely suited for infants. It is a major challenge for the industry to incorporate all the benefits provided by mother’s milk into infant formulas (Annemiek et al., 1994).

Four types of infant formula products are available.

- Infant milks
  - Whey dominant milk
  - Caesin dominant milk
- Infant formulas
  - Soya Formulas
  - Follow on milk
- Special infant formulas

These are for non breast fed infants from birth, for older infants and for infants with particular nutritional needs.
In infant milks, the protein source is derived entirely from cow's milk; whey dominated milks are the closest in composition to human breast milk. In infant formulas, the protein source is not derived entirely from cow's milk but may include soya isolates. Specially adapted infant milks are formulas (e.g. lactose free, adapted, hydrolysed protein) are also available to meet the needs of infants with special nutritional requirements.

Formula milks are specially formulated to meet the increased nutritional needs of older babies to help ensure that the recommended nutrient intakes are met. Follow on formulas are enriched with iron. They have added vitamins and minerals and contain similar amounts of energy to breast milk.

Dried milk preparations have various methods to mimic human milk. First was to add lactose. This increased the carbohydrate and also diluted the protein and inorganic constituents which are more concentrated in cow's than in human milk. Proportion of linoleic acid was increased and stearic acid decreased which made the fat more easily digested and absorbed by the young infant. Third modification was to make the product containing less sodium and phosphorus than cow's milk with higher lactalbumin : caesin ratio. Whey containing lactalbumin but not caesin is used as starter. Soluble organic constituents are removed by dialysis. Skimmed milk is then added to supply caesin and minerals, and composition is adjusted with minerals and vitamins. Lactose is added and a mixture of animal and vegetable fats. Dried milks in Britain contain Vitamin A, D and C and also iron. Some have copper and zinc (Widdowson ,1978).

In a retrospective matched pair study of 42 cases and controls during an outbreak of El tor cholera in Bahrain in 1978, the risk factors were determined. More cases were bottle fed than breastfed during the week before onset of illness. The highest attack rate of cholera (125/10000) occurred in infants in 6-11 month
age group. Various patterns of breast and bottle feeding did not determine the protection by breastfeeding (Gunn et al., 1979).

Whether formula or breastfeeding influences the functional activity of the complement system from birth to 3 months of age was evaluated by assessing haemolytic activity based on the capacity of the intact complement system to lyse sheep erythrocytes when coated with specific antibodies. Bactericidal activity of the serum against Staphylococcus aureus and Escherichia coli was used to evaluate alternative complement pathway. Sera was obtained with neonates, 1 month, 3 month old infants, breastfed or formula fed. The haemolytic capacity of serum from breastfed infants of one month was greater than that of serum from infants formula fed (Bairiga et al., 1995).

**Special infant formulas**

A large number of products is also available for infants with special needs. Products for premature or low birth weight infants are also available. Recent innovations infant formulas include the addition of antioxidants to support the immune system, long chain polyunsaturated fatty acids for cognitive development and retinal health, nucleotides to support growth, immune function and normal intestinal activity and prebiotics to aid digestion.

Reasonably safe cow's milk started to develop during the 19th century. Modern infant formulas developed during the 20th century using human milk composition as reference and cow's milk as protein source. Even with similar composition to human milk there are differences in performance between formula fed and breastfed infants. New techniques and novel ingredients within the dairy industry will contribute to minimise these differences in molecular biology allowing large scale production of recombinant human milk proteins. For formulas
containing novel ingredients with potent biological activities produced with new techniques it will be extremely important for their safety and efficacy because functional effects are not same as health benefits. (Hernell, 2011).

Acetic acid is found in higher concentrations in breast fed than formula fed infants. Degradation of mucin starts later in breastfed than in formula fed infants. The conversion of cholesterol to coprostenol is also delayed by breast feeding (Orrhage et al., 1999).

In a population based case control study in an urban area of North India the role of feeding breast milk, bovine milk or infant formula during diarrhoea in protecting against or increasing persistence of episodes was investigated. Exclusive breastfeeding was associated 16.5 times lower odds in favour of developing persistence of an episode. Bovine milk in addition to breast milk was 2.5 times in favour of developing persistence to acute diarrhoea. Infants receiving bovine milk and no breast milk, odds was 11.1%. This study indicates that promoting exclusive breastfeeding may reduce persistence of diarrhoea over and above its effect in decreasing the incidence of acute diarrhoea. In urban areas the use of artificial formula milk supplements increases the risk of developing persistent diarrhoea (Sazawal et al., 1992).

Infant formulas play a major role in the field of newborn and infant artificial feeding, reducing the gap with maternal feeding. In the last decade, milk formulas became more and more similar to human milk starting from cow's milk, obtaining a composition adequate to the digestive and nutritional requirements of the new-born and of the infant. Major differences between human and cow's milk have been corrected by dilution and skimming. Intolerance or allergy to cow's milk, the substitution of the cow's milk based formula with hydrolysed or soy
formula is required. Breast milk represents the best choice for the nutrition of the newborn (Piacentini et al., 1995).

Infant feeding practices - when mother decide to breastfed or formula fed are necessary to develop programs to effectively promote breast feeding. Surveys showed that 85% to 92% of mothers decided on a feeding mode. From 1976-1980 infant feeding practices changed as increase in incidence and duration of breastfeeding, decrease in new-born infants receiving formula, increase in use of formula rather than cow’s milk when breast feeding is discontinued early, and later introduction of supplementary foods for both breastfed and formula fed infants (Sarett et al., 1983).

Microbial contamination of powdered infant formula (PIF) is known to cause gastrointestinal infections in infants. Of concern is intrinsic contamination of the formula as well as extrinsic contamination from inappropriate handling or ineffective disinfection (Elizabeth et al., 2009).

Bottle fed (Non breast fed) infants had an incidence of diarrhoea more than three times higher than exclusively breastfed infants. Of particular interest is the finding that infants colonized with enterotoxigenic *Escherichia coli* producing heat labile toxin (LT - ETEC) have a lower risk of diarrhoea when breastfed, specially by the amount of pathogen specific secretory antibody the infant is receiving per day via the mother’s breast milk (Long et al., 1994).

Risk of diarrhoea was associated with infant feeding mode in both urban and rural samples. Diarrhoeal morbidity was higher in rural infants than urban areas due to lack of awareness of oral rehydration therapy (Popkin et al., 1990).

Risk of diarrhoea was significantly associated with infant feeding mode in the expected direction. (Brown et al., 1987).
In a study conducted in urban South Indian families, at 6 weeks, 2% of the exclusively breastfed infants had diarrhoea compared with 24% of partially breast fed infants. At 14 weeks, 0% of the EBF (exclusively breast fed) infants had diarrhoea, compared with 7.5% of the PBF (partially breast fed) infants (Unni et al., 1988).

35% of the exclusively breastfed infants had diarrhoeal disorder during the first 24 months of life compared with 76% of the partially breastfed infants and 74% of the formula fed infants in a study in Nigerian infants (Scott et al., 1986).

Bacteriological quality of 244 infant feeding-bottles and 61 teats were studied from January, 1989 to November, 1989. Samples were collected from feeding-bottles of babies who were brought by nursing mothers to clinics and hospitals for varying complaints. In a rural US community based study, breastfed infants had fewer diarrhoeal episodes than did bottle fed infants for the first 6 months (Paine et al., 1982). The superior quality of breast milk, and continual teaching of bottle feeding mothers on better handling of feeding bottles and feeds should be constantly emphasized. Teaching mothers on proper handling of feeding utensils accompanied by regular home visiting of health workers to observe hygienic condition of feeding utensils and home environment may improve the prevailing condition. Of the 244 bottle-content samples, 144 (59%) harboured more than 2 x $10^6$ colonies of bacteria per ml. of samples. The predominant bacterial isolates both from bottle-contents and teat-swabs were the coliform group of bacteria, such as *E. coli*, *Enterobacter spp.*, *Klebsiella spp.*, and *Citrobacter spp.* Enteric pathogens such as enteropathogenic *E. coli*, *Shigella spp.*, *S. aureus*, and *Bacillus cereus*, were also isolated from some samples. In some cases enteropathogenic *E. coli* (EPEC) serotype were isolated both from feeding utensils and from the stools of the bottle-fed babies. Neither the different handling care of bottles used in the studied population, nor the educational status of the nursing
mothers made a significant difference in the bacteriological quality of the infant feeding bottles in terms of the total bacterial counts per ml of the bottle-contents. Preparation of food in advance of need combined with improper storage and inadequate cooking, and poor hygiene could be factors that resulted in a highly contaminated bottle contents. Further and well controlled community based, longitudinal studies are also needed if we are to reduce gastroenteritis in babies as a result of using feeding bottles.

In a cross-sectional analytical study from July to August 1988 on factors influencing bottle feeding practice in infants at the Well Child clinic was done to define factors influencing bottle feeding practice in infants. Of the 264 cases studied, 85 infants aged 0-3 months, 10 infants (11.7%) were exclusively bottle fed, 35 (41.2%) bottle and breastfed and 40 (47.1%) exclusively breastfed. The practice of bottle feeding seemed to increase with age. In 85 infants of 7-12 months, 28 (32.9%) were bottle fed, 29 (34.2%) breast and bottle fed and 28 (32.9%) breastfed, 30.7% bottle fed infant mothers. One child families preferred bottle feeding (30.7%). Higher the income, the more they tended to give bottle feeding more (30.7%). 176 were nourished babies of which 74 were (42.1%) breastfeeding, 75 (42.6%) breast and bottle feeding, 54 well nourished babies aged 7-12 months, 15 infants (27.7%) were breast fed. 20 (37.7%) breast and bottle fed and 19 (35.2%) bottle fed (Lukman et al., 1991).

Bacteria that cause diarrhoea can be classified according to the way in which they interact with the gut mucosa, as summarized in a review groups were suggestions as follows:

1. Mucosal adherent, entero toxin producing
2. Brush border effacing
3. Mucosal invading and intra-epithelial cell proliferating
4. Mucosal translocating and proliferating in lamina propria and mesentric lymph nodes

5. Mucosal translocating leading to generalized infection (Levine et al., 1987).

The differential diagnosis of chronic diarrhoea is very extensive. Regardless of specific etiology, increased intestinal secretion inhibition of active ion absorption and abnormalities of mobility are seen (Krejas and Fordtran., 1978).

**CLINICAL FEATURES OF GASTROENTERITIS**

Profuse diarrhoea in infants and children can cause serious fluid, acid base and electrolyte disturbances and these changes can progress with frightening speed to produce circulatory failure.

The mechanism of EPEC attachment is unknown, but the original work of McNeish and co-workers implicating a 60 - megadalton plasmid in this process has been confirmed (Baldini et al., 1983). Cloning and mapping studies are under way to identify genes that encode for the proteins involved.

The infant feeding practices and their relationship with diarrhoea in Huascar, Peru were studied. Infant feeding mode was significantly associated with the risk of diarrhoea. Infants < 6 months of age given non-nutritive liquids only in addition to breastmilk had a relative risk factor of 2. Infants < 6 months of age given breastmilk and artificial milk had a relative risk of 1.6 to 2.4. Infants <6 months of age given breastmilk and solids had a risk of 2.6 to 3.4. Infants < 6 months of age who were not breastfed had a relative risk of 3.4 to 5.5. 6-11 months old infants who were partially breastfed seemed more protected from diarrhoea than infants who received no breastmilk (Brown et al., 1989).
In a comparative study of morbidity and mortality of breastfed and bottle-fed Nigerian infants stated that the risk of diarrhoeal illness was lower in exclusively breastfed than in partially breastfed or bottle-fed infants. 76% of the partially breastfed infants and 74% of the formula-fed infants suffered from diarrhoea when compared to 35% of the exclusively breastfed infants (Scott-Emuakpor et al., 1986).

**Types of diarrhoea**

Diarrhoea in infants may be due to a variety of causes but it has been rather customary to classify them into.

1. Dietetic
2. Parenteral
3. Enteral

Dietetic type of infantile diarrhoea has decreased and parenteral type of diarrhoea is due to infection elsewhere such as rhinitis, pharyngitis, otitis media, mastoiditis, septicaemia, pyaemia.

68 bottle fed babies under 9 months of age with mild acute gastroenteritis were observed to evaluate current feeding regimens following acute gastroenteritis in infancy. An enteric pathogen was detected in eight of this group and cow’s milk protein intolerance in three (one from each feeding group). Many infants (6/24) refused to take whey hydrolysate formula (Armitstead et al., 1989).

In the enteral group there are two important types of diarrhoea - one in which a definite cause can be ascertained (e.g.) the dysentries (by shiga, flexneri, some organisms) which are associated with diarrhoea with blood and mucus in the
stools, vomiting etc., and the other group where no cause is found, and which is labelled 'acute gastroenteritis' of infants the non specific group.

209 children in the age range of 4-24 months from Dhaka Hospital, Bangladesh were studied to assess the relationship of dietary pattern with nutritional status, micronutrient status and bacterial pathogens. Feeding practices were recorded. Nutritional status was assessed with standard anthropometric measurements, diarrhoeal pathogens were isolated by microbiological assay. 12% of the children were exclusively breastfed, 10% were formula fed, 37% were breast milk and formula fed. Breastfeeding was 67% in infants below 5 months. 16% of illiterate mothers exclusively breastfed their babies compared to 7% of educated mothers. 15% of poor mothers breastfed their infants when compared to 7% of middle class mothers. Pathogens were isolated from 67% of infants. Exclusively breastfed (12%) infants had fewer bacterial pathogens than non–breastfed (25%) infants (Roy et al., 2009).

Effect of not breastfeeding on the risk of diarrhoeal and respiratory mortality in children under 2 years of age in Metro Cebu, The Philippines was studied in longitudinal study of 9942 children in metro Cebu from 1988-1991. Examined the effects of not breastfeeding on mortality due to diarrhoea in children under 2 years of age. Failure to initiate breastfeeding in the first 6 months of life or ceasing to breastfeed had a greater effect on diarrhoeal mortality (8-10 fold increase). These findings underscored the importance of promoting breastfeeding especially during the first 6 months of life (Yoon et al., 1996).

In studies in UK, the independent effects of solids and breastfeeding on the risk of hospitalisation for infection was assessed. For both diarrhoea and lower respiratory tract infection (LRTI), the monthly risk of hospitalisation was lower in those receiving breast milk compared with formula fed (Quigley et al., 2009).
In their study emphasised that exclusive breastfeeding, bottle feeding, ever breastfed were 44.9%, 67.8%, 31.5% and 99.4% respectively. The significant positive independent association of exclusive and partial breastfeeding were infant's present weight, breast milk as first food, positive independent association for exclusive and partial breastfeeding were infant's present weight, breast milk as first feed. Breastfeeding propagation plays a key role in promoting exclusive breastfeeding (Sachdev et al., 1995).

451 mothers were studied in two University hospitals in Tehran for feeding patterns. 98% had breastfed their babies during the first few hours of birth. 83% had followed full breastfeeding for 6 months with no bottle feed introduction. 6.5% of infants were fed on breast and bottle concomitantly. 10.5% had breastfeeding discontinued before 6 months and bottle fed. Caesarean delivery and infant hospitalisation of the infant during neonatal period was also associated with a higher rate of bottle feeding compared normally delivered babies (Shiva et al., 2003).

During the first year of life, the incidence of diarrhoea was twice as high among formula–fed infants as among breastfed infants. Diarrhoeal morbidity during the second year of life did not differ between the two groups. Breastfeeding protects against diarrhoea even in highly educated populations (Dewey et al., 1995).

The risk factors for diarrhoeal disease incidence in early childhood, was studied in a community cohort study in Guinea-Bissau. Bivariate analysis show that compared with exclusive breastfeeding, both partial breastfeeding and no breastfeeding are significant risk factors for diarrhoea. In multivariate analysis, the comparison between exclusive breastfeeding and no breastfeeding was significant. Breastfeeding is an effective modifier of other risk factors for diarrhoea and there
was no association between maternal education and diarrhoea as long as the children were breastfed. Weaned children had strong and independent associations among socio-economic, demographic and environmental variables. Promotion of breastfeeding is a major preventive measure against diarrhoea in developing countries (Melbalk et al., 1997).

A cross sectional study in Accra, Ghana was done on 376 women with 0-6 months old infants to assess factors associated with exclusive breastfeeding (EBF). Of the 99.7% of mothers who were currently breastfeeding only half (51.6%) of them exclusively breastfed their infants. 98% had heard about EBF and 85.6% of them planned to EBF on delivery. 'Since birth' EBF and planned EBF on delivery was associated with higher EBF rate. Hospital delivery was associated with higher EBF rate. Rented accommodations and family houses affected women who breastfed (Aidam, et al., 1990).

209 women having infants 4 months or less living in Hapira, Brazil were interviewed to assess factors associated with infant feeding practices. Exclusive breastfeeding on the first day at home was 78.1% and infant formula fed was 11.6% (Andi et al., 2005).

235 Asian and 462 non Asian first time mothers were compared for infant feeding practices from 2 Sydney hospitals and at 6 and 12 weeks after birth, Non - asian women vs Asian women (83% vs 87%) at 6 weeks (60% vs 61% ) or 12 weeks (51% vs 56% ). Asian women breastfed their babies partially than Non-Asian women (Dahlen et al., 2010).

Exclusive breastfeeding (EBF) upto 6 months was studied in urban slums of Gwalior, India. From Nov 2005 to July 2006, 279 infants aged between 6 and 11 months were investigated. Only 11 (3.8%) mothers knew that EBF should be
continued till 6 months and 22 (7.8%) practiced EBF. Early breastfeeding initiation, mother's education and Ante natal clinic visits were associated with EBF probability. Women with regular contact with health facilities were breastfeeding. Mothers who start breastfeeding early are more likely to exclusively breastfeed their infants (Tiwari et al., 2009).

234 mothers were studied to examine the influence of socio demographic factors on infant milk feedings and the protective role of breastfeeding against infections. Only 31 (135) mothers practiced exclusive breastfeeding (EBF) and 133 (57%) used exclusive infant formula feeding (EIF) (Chye et al., 1998).

Six villages of Panchkula district, Haryana, a national family health survey revealed lower breastfeeding rates (16.9%). Time of initiation of breastfeeding, duration of EBF were assessed. Out of the 77 mothers, 30% and 10% were exclusively breastfed till 4 and 6 months of age. 39% of mothers had satisfactory breastfeeding knowledge. EBF/PBF are suboptimal among rural North Indian mothers (Kishore et al., 2009).

In a cohort study in Durango, Mexico for 3 months, infants who were not breastfed were at a greater risk having acute diarrhoea during early infancy despite unfavourable social and economic conditions. Protection conferred by breastfeeding is stronger when home conditions are poor (Macias Carrillo et al., 2005).

A cross sectional study was conducted in rural areas in the western region of Ethiopia, Gama-Gafa to determine the extent of protection from breastfeeding. Out of the 331 infants, 217 (66%) were exclusively breastfed and 114 (34%) were partially breastfed. Out of the exclusively breastfed (n=217), 25 (12%) had
diarrhoea. Breastfeeding tends to diminish with urbanisation and should be promoted to control infantile diarrhoea (Ketsel et al., 1990).

Breastfeeding practices were described and assessed the socio-demographic factors associated with selected breastfeeding indicators. 97.6% infants had ever been breastfed, only 46.1% had initiated breastfeeding within the first hour of birth. 78% were currently breastfed (24 months), 30.7% were exclusively breastfed (6 months), 12.5% were bottle fed (12 months). Exclusive breastfeeding was significantly lower in rural west region compared to the urban region and among rich households than poor (Senareth et al., 2007).

A study was conducted to determine the incidence of diarrhoea and to assess associated factors in children younger than 40 months living in 2 slums of Salvador, Brazil. 84 children were selected randomly. 232 episodes were identified, incidence rate of 2.8 episodes /child/year. In average each child suffered 11.1 days of diarrhoea per year. The highest incidence rates were found among children under one year old (Melo et al., 2008).

A cross sectional study was conducted involving children under 5 years of age in three places on S. Luiz island i.e., Vila Palmeira, Anjo da Guarda and S. Jose de Ribamar. The prevalence of diarrhoea was highest in May, 1986 and November, 1987. Highest prevalence occurred in S. Jose de Ribamar. High prevalence of diarrhoea was seen among children of 6-11 months. Infant mortality in May 1986 was 44/1000 in children under 1 year of age and 12.8 in children under 5 years old (Campos Gde et al., 1995).

The prevalence of breastfeeding in Lebanon was studied over 10 months as a cross sectional design by. One-third of the mothers stated that breast milk was the first food after birth. 55.9% started breastfeeding within few hours after birth,
4.6% never breastfed. Rural mothers practised exclusive breastfeeding for longer duration. 95.4% of mothers initiated breastfeeding, an ecological perspective on intervention aimed at women and their social support system to improve duration of breastfeeding (Batal M et al., 2006).

A cross sectional study was conducted for investigating infant feeding practices in Mangochi district, Malawi. Early breastfeeding was practiced among 68.2% of rural and 63% of the semi urban mothers. Exclusive breastfeeding rates in the sample at 2, 4 and 6 months were 39.1%, 27.5% and 7.5% respectively. At 4 months, exclusive breastfeeding was higher in semi urban areas (46.8%) than in rural (4.7%) group. Semi urban mothers practice optimum breastfeeding and health facilities have an important role in its promotion (Kamudoni et al., 2007).

Delayed initiation of breastfeeding and neonatal mortality (2-28 days) were reported in rural Ghana. This prospective observational study of 10942 breastfed singleton infants between July 2003 to June 2004. 140 neonates died from day 2 to day 28: 93 died of infection and 47 of non infectious causes. Death as a result of infection with delay in initiation of breastfeeding from 1 hour to day 7 was associated with a 5.7 fold adjusted risk of deaths as a result of infectious diseases. This study provides the first epidemiologic evidence of a causal association between early breastfeeding and specific neonatal mortality in infants (Edmond et al., 2007).

Infant feeding practices determine the nutritional status of children and are associated with household socio-economic and demographic factors. In a cross sectional study in households, a total of 805 child-mother pairs were randomly sampled. Breastfeeding was universal in the study area. 22% of mothers initiated breastfeeding within one hour whereas 44% initiated 3 hours after delivery. Prelacteal use was high. 41% of infants were exclusively breastfed for 6 months and
58% of infants (6-11 months) received complementary feeding at 6-9 months of age. Timely breastfeeding and exclusive breastfeeding was more among scheduled castes and scheduled tribes mothers. Early initiation of breastfeeding (within an hour of birth) are associated with community, type of family and mother's education. Efforts are needed to promote breastfeeding (Meshram et al., 2012).

Breastfeeding was practiced by 99% of the mothers. Dietary recall since birth showed 7% and 8% practiced exclusive breastfeeding by 3 and 6 months. The cross-sectional study on infant feeding practices performed in Mbake district, Eastern Uganda in 2003. 30% and 3% practised predominant breastfeeding and not started complementary feeding at the same time. Dietary recall since birth might be an alternative to monitor infant feeding practices in poor settings. This study emphasised the need for improving infant feeding practices in Eastern Uganda (Engebretsen et al., 2007).

Infant and young child feeding practices are different among communities. a cross-sectional study of 360 mothers in Chepang communities of Makwanpur District was undertaken. Feeding practices of mothers were found better than their knowledge about early initiation of breastfeeding.81% for exclusive breastfeeding and 90% for introduction of complementary foods at the age of six months. Feeding practice was associated with health and socio-economic determinants (Subedi et al., 2012).

Breastfeeding as the most appropriate form of infant nutrition for the first 6 months of life, this impact has been softened by the favourable suitability of artificial feeding. Infant feeding with artificial formulas increases the risk of short and long term morbidity and mortality. Breastfeeding promotion challenges health professionals in rural Australia to adopt the axiom that "Breast is best and bottle is a last resort"(Hughes, 1996).
150 children from birth to 1 year were investigated in a study to estimate the value of breast milk as a solution to the infant nutrition problem. 15% of the children were exclusively breastfed. 44 were breastfed plus cow's milk fed and 40.6% were fed only cow's milk. 33% of the breastfed babies, 44.4% of mixed fed and 47% of cow's milk fed were malnourished (Wright Mdale et al., 1986).

In their study of children aged 12-24 months in Guinea-Bissau it is stated that weaning was associated with the risk of diarrhoea. The relative risk of diarrhoea was 1.41 for weaned children compared with breastfed children. The mean duration of diarrhoea was longer in weaned children than in breastfed children. A similar increase in risk of diarrhoea was found when the rate and duration were compared 1 month before and 1 month after weaning for each child. The association between prolonged breastfeeding and poor nutritional status is explained rather than a negative effect of breastfeeding on child growth. The results of the study, independent of the age of weaning, show that the protective effect of breastfeeding on diarrhoea is unlikely to be affected by unknown factors associated with both infant feeding practices and risk of diarrhoea (Melbak et al., 1994).

The social factors were studied in the prevalence of diarrhoeal diseases in under-five children in urban and rural areas of Saudi Arabia. The prevalence of diarrhoea was 18.5%, 23.3%, 17.7% and 13% for exclusively breastfed, breastfed and bottle fed infants, bottle fed only and receiving other food only respectively. Breastfeeding and risk of diarrhoea are age related. Breastfeeding is more common among uneducated rural mothers, and most women who only bottle fed are educated and employed. Prevalence of diarrhoea is higher in infants in the bottle category than in other categories (Al-Mazrou et al., 1995).
The rural environment provides a situation in which breastfeeding can flourish. Certain attitude and lifestyle is needed if breastfeeding is to be successful. Urbanisation and the change in lifestyle, breastfeeding becomes difficult. Successful breastfeeding needs motivation, change in attitude of urbanised wives make it unlikely. Benefits of breastfeeding is widespread, the motivation to breastfeed is not. Measures should be taken to motivate mother and family (Tan et al., 1983).

Responses to focus groups showed that ignorance about breastfeeding and belief that mother's milk was inadequate and health system acted as barriers to women breastfeeding their infants up to the age of 4 to 6 months. In a study in Chengdu, China, 363 mothers were surveyed. Mothers agreed that breast milk is the best food for their infant up to the age of 4 to 6 months. Only half of the mothers breastfed for longer than one month. The researchers conclude that Chengdu health workers should teach mothers about breastfeeding (Guldan et al., 1995).

Breast milk is recommended universally as the preferred infant nutrition source. It is superior in nutrient and immunologic properties. Breastfeeding can be successful when nursing on demand, Prevention of sore nipples and convenient access to medical advice. Mothers requiring bottle feeding, cow's milk based formula is preferred. Protein hydrolysate formula should be used in infants who cannot tolerate cow's milk based on soy based formulas. Low iron formulas and whole cow's milk should not be used during the first year. Breastfed infants do not require vitamin supplementation. Soy based formulas are lactose free, they may be tolerated by infants who are allergic to cow's milk protein (Spencer, 1996).

A clinical and prospective epidemiological study was conducted of acute diarrhoea in children under 5 years of age at King Mongkut Prachomklao
Hospital. Of the 105 cases of acute diarrhoea patients, 64 patients had causative pathogens. Common bacterial pathogens were *Escherichia coli* (14.1%), *Campylobacter jejuni* (14.1%), *Shigella* (12.5%) and *Salmonella* (3.1%). Mixed infections were reported in 31.3% of patients. Younger patients had a higher percentage of identifiable pathogens (66.7%). Most cases had at least mild to moderate dehydration, so oral rehydration solution (ORS) was successfully given in only 31.4% of patients. Good hygiene behaviour to reduce diarrhoeal episodes should be emphasised and the use of ORS should be promoted to reduce diarrhoea severity (Suwetano *et al.*, 1997).

Acute diarrhoea due to bacterial infection can be classified into three groups based on pathogenic mechanisms. The first group exemplified by *Cholera vibrio* and enterotoxigenic *E.coli*, produce diarrhoea by multiplying in the small intestine and secreting enterotoxins which cause profuse search of fluid and electrolytes. The bacteria do not invade the bowel mucosa, which appears histologically normal. Systemic symptoms like fever are minimal or absent. The second type is represented by *Shigella* and enteroinvasive *E.coli*. They invade the bowel. The pathology is initially in the small intestine, when the patient develops abdominal cramp, mild fever and watery diarrhoea. In a day a two, the large bowel is invaded, with colitis leading to urgency, tenesmus and bloody mucoid stool typical of dysentry. The third group is identified as *Salmonella*. The bacteria penetrate the gut mucosa and reach the lamina propria where they set up inflammation. Bacteraemia and systemic symptoms such as fever are very common. In general, profuse watery diarrhoea is seen in enterotoxin infections such as cholera, as well as in rotaviral diarrhoea. Invasive diarrhoea leads to liquid stool rich in cellular exudate.

Hypernatraemic dehydration remains an important and serious complication in infants with gastroenteritis. 67 children aged 18 days to 18 months
admitted with hypernatraemic dehydration caused by acute gastroenteritis. 5 hypernatraemic infants (7.5%) were breastfed compared with 40 (60%) isonatraemic controls. Artificial milk feeding particularly the use of evaporated cow's milk powder is a predisposing factor for hypernatraemia in infantile gastroenteritis (Abu-Ekteish *et al.*, 2002).

500 Brazilian infants < or = 12 months with diarrhoea and 500 age matched controls, breastfeeding infants , 6months old were protective. Breast feeding was protective against EPEC infections (Blake *et al.*, 1993).

The water supply and sanitation project in a rural area of Bangladesh was evaluated. Diarrhoeal morbidity in 0-4 year old children was recorded . Persistent diarrhoea was due to *Shigella species*. 16% of all episodes was classified as persistent and seen in the age group of 0-5 months (Huttly *et al.*,1989).

When compared with exclusively breastfed infants, infants who were exclusively formula fed had an 80% increase in their risk of developing diarrhoea. The risk of developing diarrhoea increases as the amount of breast milk an infant receives decreases. The incidence of diarrhoeal illness among breast fed infants was half that of formula fed infants in the year of life (Dewey *et al.*,1995).

In a study in primary health care centres in Riyadh, Saudi arabia, prevalence of diarrhoea in children less than 2 years was estimated .Diarrhoea was more common in children over 6 months of age.

A retrospective study of infants and children admitted to two paediatric hospitals was conducted in Baghdad city during 1990-1997. 14.9% suffered from diarrhoea, 55.5% were under 1 year of age. The peak incidence was at 3- 6 months . In 97% of cases only one pathogen was identified, enteropathogenic *Escherichia*
coli (EPEC) being the most frequently isolated organism isolated from 13 % of patients (Tawfeek et al., 2002).

Persistent diarrhoea is the most common disease in developing countries. Children of age below 6 months were tested, enteropathogenic *Escherichia coli* (EPEC) was isolated. Etiologic research was positive in 57.1% of the cases. EPEC was isolated from 42.9% of cases followed by *Shigella* (9.5%) and *Salmonella* (5.9%). 25 were below 6 months old. From the isolated EPEC (35/189), 26 (74.3%) belonged to 0111 serogroup (de Andrade et al., 1998).

**AETIOLOGY OF DIARRHOEAL DISEASES**

A large number of micro-organisms are now known to cause diarrhoea. Until recently, the causative agents could be identified in only 10-20% of diarrhoeal illnesses. In the last decade, additional pathogens have been recognised so that now, under optimal laboratory conditions, aetiological diagnosis can be established in 80% cases.

**A. BACTERIA**

1. *Vibrio*

   a. *V. cholerae*

   *V. cholerae*, classical as well as Eltor biotypes, cause cholera, the most spectacular of diarrhoeal disease. The *vibrio* enters the intestine through food or drink. The infective dose is large about $10^8$ cells. The site of infection is the small intestine, primarily the pathogenicity depends on the ability of the *vibrio* to adhere to intestinal epithelium and colonies in the gut, as well as on the production of the toxin.
Vibro cholerae serogroup 0139 was first reported to be responsible for cholera in India in 1992. Its appearance and rapid spread to various parts of India and to other countries including Bangladesh and Thailand has been reviewed. (Jesudasan and Jacob John, 1994). The isolation of V. cholerae 0139 in 1993, its quiescence till 1997 followed by its re-emergence is reported by Rant et al. (1999).

Cholera is endemic in Bangladesh, West Bengal and many other parts of India. In endemic areas it is predominantly a disease of children when it appears in non endemic areas. It affects all ages, most commonly adult males. In both endemic and epidemic situations, cholera exhibits seasonal patients, which vary in different areas.

Cholera can in varying grades of severity, from a rapidly fatal disease to a mild diarrhoea. A symptomatic infection is common with el tor vibrios.

b. Non-cholera (NAG) Vibrios

These vibrios resemble cholera vibrios except for being non agglutinable with 0 group antiserum. The pathogenic status of NAG vibrios is not clear. Some of them have caused out breaks of cholera like disease. While most of them appear to be non-pathogenic they have been frequently observed in natural water sources and in normal stools. A few strains have been shown to produce a heat labile enterotoxin resembling the cholera toxin.

c. V. parahemolyticus

V. parahemolyticus was first isolated in Japan in the 1950's from outbreaks of food poisoning, this halophilic vibrio has subsequently been demonstrated in the coastal waters and several countries. Several O and K antigenic types of the
vibrio exist, but there is no association with pathogenicity and any particular serotype. Hemolysis on special high salt agar (Kanagawa phenomenon) has been claimed to be useful in differentiating between pathogenic and non pathogenic strains.

2. **Escherichia coli**

*E.coli* appears to be the most versatile of diarrhoeagenic agents. Atleast four mechanisms of pathogenesis have been identified in *E.coli* diarrhoea, a different set of strains being responsible for the different types of pathogenesis.

Diarrhoeagenic strains of *E.coli* are marked by their ability to adhere to the epithelium of the bowel and colonize it. Many strains have additional virulence properties, such as the ability to produce toxins. Differences in mechanisms of virulence led to classification as enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxin (or shigalike toxin) producing (VTEC or SLTEC) and enteroaggregative (Eagg EC).

MC Neish *et al.* (1975) first showed that *E.coli* of EPEC serotype could adhere to human fetal intestine. This property was conferred by a 60megadalton plasmid (Williams *et al.*, 1978). Adhesion of EPEC to human intestine *in vitro* was confirmed by electron microscopy of intestinal biopsies from infected infants (Rothbaum *et al.*, 1982).

Typical and Atypical EPEC seem to constitute two groups of distinct organisms that have in common the LEE pathogenicity . Atypical EPEC have become a more frequent cause of diarrhoea in industrialised countries .Typical EPEC serotypes have been associated with outbreaks of infantile diarrhoea (Luiz *et al.*, 2002).
Indonesian children less than 5 years of age were studied to determine the prevalence of diarrhoea caused by enterotoxigenic *E.coli* (ETEC) and other bacterial enteropathogens. ETEC was the second most frequent cause of diarrhoea isolated from 16 of 194 (8.2%) of patient's stools. Highest prevalence was in infants 12 to 23 months of age (17.9%) (Subekti *et al.*, 1993).

A community based prospective study was conducted from Dec 1993 to March 31, 1994 in Indonesia in children less than 5 years. Enterotoxigenic *E.coli* was identified in diarrhoea stool and isolated from 19% of 340 episodes of diarrhoea. 61% of ETEC produced heat labile toxin (LT) only. Age specific incidence rates of diarrhoea was among children aged 0-1 (77%). ETEC was isolated from 26% of children 9-1 years of age versus 53% for children 2-3 years of age (Orndorff *et al.*, 1996).

In a study carried out on 1600 rectal swabs from children under 5 years of age admitted at the health center in Islamshahr, Tehran province, Islamic Republic of Iran during 1998-99. Specimens were examined for various bacterial pathogens. Isolation rates were enteropathogenic *Escherichia coli* 6.8%, *Shigella* spp 3.4%, *Salmonella* spp 2.9%, *Campylobacter* spp 0.9%, *Yersinia* spp 0.7%. The isolation rate was highest in summer (Dallal *et al.*, 2006).

107 diarrhoeal cases were studied and isolates from 71 (66%) of which 60 (85%) were due to single agent and remaining 11 (15%) were of mixed infections. Enterotoxigenic *Escherichia coli* (ETEC) was isolated from 65 cases. *Salmonella* spp and *Shigella* spp were isolated. Higher isolation rates of ETEC was seen. The infection rate was higher for 0-2 year of age as compared to 3-5 year age group (Samuel *et al.*, 1997).
In a birth cohort (n=252) in rural Bangladesh the incidence of aetiology specific diarrhoea and the pathogenicity of infectious agents were determined. Out of 1750 stool specimens, 58% had an infectious agent. The most commonly isolated pathogens from all specimens were enterotoxigenic *Escherichia coli* (ETEC), Enteroadherent *E.coli* and *Shigella*. Aetiology specific infections were associated with acute episodes. The isolated enteropathogens were essentially the same as those found in other rural areas (Hasan *et al.*, 2006).

*Escherichia coli* emerged as a cause of infantile diarrhoea in 1865. Currently there are four pathogenic categories of diarrhoegenic *E.coli* and recognised Enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC). ETEC and EPEC are the main causes of paediatric diarrhoea especially in developing countries. ETEC strains are characterised by their production of well defined heat labile and heat stable enterotoxins. EPEC strains have been identified by serotyping only, recent laboratory and clinical investigations have shown that these strains constitute a distinctive group of pathogenic micro-organisms exhibiting adherence to epithelial cells and producing pathognomic histopathologic changes in naturally infected children (Robins-Browne *et al.*, 1987).

In a study conducted in Galicia, north Western Spain, the role of enterotoxicogenic *Escherichia coli* (ETEC) in infantile diarrhoea, 482 children with diarrhoea were investigated between 1985 and 1988. *Salmonella spp* (12.8%) were the mostly isolated, followed by ETEC (3.9%), *Shigella spp* (0.9%) and *Campylobacter jejuni* (2.3%) and *Yersinia enterocolitica* (0.5%). In infants with diarrhoea who were under 1 month of age (26.5%). ETEC were isolated more frequently than from older diarrhoeic children (2.2%). Seventeen ETEC isolates produced heat stable enterotoxin only, four produced only heat labile enterotoxin
and two produced both toxins. The results suggest that ETEC is an important cause of neonatal diarrhoea in this part of Spain (Blanco et al.,1991).

648 patients in Sweden with acute diarrhoea were investigated for enterotoxigenic strains in stool cultures. 74 strains were isolated from 28 patients. Only three of enterotoxigenic E.coli (ETEC) belonged to classical EPEC. None of the 67 isolates of EPEC produced heat labile (LT) enterotoxin. LT and ST strains were common in stool isolates of Ethiopian children but rare among Swedish children with acute infantile diarrhoea (Wadstorm, 1978).

Preliminary analysis of enterotoxin production in rural Ghana showed 11% LT-ST, 9.5% LT+ST and 7.5% EPEC. In urban Ghana the results showed 10.9% LT-ST, 5.9% LT+ST, 1.6% LT+ST+ and 6.5% EPEC. 14 serotypes of EPEC were isolated in the urban area whereas 6 serotypes in rural areas. The four recognisable classes of Escherichia coli that causes diarrhoeal disease in humans are EPEC, ETEC, EIEC and EHEC (Agbodaze et al., 1988).

Enterotoxigenic Escherichia coli (ETEC) was assessed as etiologic agent of diarrhoea in infants aged less than 6 months in a hospital in Calcutta, India. ETEC was isolated from 26 (11.9%) cases of 218 cases examined. Of these 17 cases, ETEC was the sole infecting pathogen. Of the 26 ETEC strains detected, 15 (57.7%) produced heat labile toxin (LT), only 8 (30.8%) produced heat stable (ST) toxin only and the remaining 3 (11.5%) produced LT and ST. All ETEC isolates were multiple drug resistant (Ghosh et al., 1996).

A group of children with endemic diarrhoea was admitted to Dhaka Shishu hospital in Dacca, Bangladesh. EPEC was detected in faecal samples of 25% of 104 cases. This study supported the concept that EPEC may be the main cause of endemic diarrhoea in Bangladesh (Moyenuddin et al., 1985).
The proportional hazards analysis of diarrhoea due to Enterotoxigenic Escherichia coli and breastfeeding was studied in a cohort of urban Mexican children. The incidence of diarrhoea in non breastfed infants fed only formula was 3 times higher than exclusively breastfed infants and twice as high as partially breastfed infants. Depending on the amount of pathogen specific secretory antibody the infant is receiving per day via the mother’s breast milk, the infants colonised with enterotoxigenic *Escherichia coli* producing heat–labile toxin (LT-ETEC) have a lower risk of diarrhoea when breastfed. The study shows that the introduction of complementary foods increases the risk of pathogen colonisation, and that the expression of infection depends on the amount of protective antibody the infant receives via breastmilk (Long *et al.*, 1994).

513 Venezuelan infants with diarrhoea were studied to evaluate the epidemiological significance of Hep2 cell adherent *Escherichia coli* isolates in diarrhoeal disease and to determine the prevalence of enteropathogenic *E.coli* and their correlation with O:H serotypes. *E.coli* isolates exhibiting localised and aggregative adherence in the Hep2 cell assay were most frequently isolated from the patients (8.5% and 26.9%). The difference was significant for the group 0-2 months of age but for older infants. Regardless of age, *E.coli* isolates with diffuse adherence were found at same frequencies in both the patients and controls. These findings confirmed the pathogenic role of *E.coli* with localised and aggregative adherence in diarrhoea as well as the epidemiological importance of O:H serotyping for characterising localised adhering *E.coli* (Gonzalez *et al.*, 1997).

1082 stool specimens of pre-school children with diarrhoea were investigated for the presence of enterotoxigenic (ETEC) and enteropathogenic *Escherichia coli* (EPEC). ETEC was found in 114 (10.5%) EPEC was isolated from stools of 85 (7.9%). 19 EPEC strains showed localised adherence to Hep-2 tissue culture cells. *Shigella* species were isolated from 17.5% of children. ETEC
was associated with acute diarrhoea in pre-school children. EPEC was associated with sporadic diarrhoea in 0-2 years of age in investigated localities (Cobeljic et al., 1989).

Enteropathogenic strains of *Escherichia coli* (EPEC) that caused 10 outbreaks of infant diarrhoea in the UK between 1968 and 1986 were studied. The strains isolated from the sporadic cases of diarrhoea in the UK were similar to hybridisation and adhesion to those isolated from sporadic cases of diarrhoea in developing countries (Scotland et al., 1989).

In a study of 1082 patients, enteropathogenic, enterotoxigenic and enteroinvasive *Escherichia coli* were isolated from 52 (4.8%) in the Lagos University teaching hospital, Lagos, Nigeria between Oct 1979 and March 1981. Of the 52 strains isolated, 35 (67.3%) were enteropathogenic, 12 (23.1%) were enterotoxigenic and 5 (9.6%) were enteroinvasive *E. coli*. Diarrhoea associated with enteropathogenic *E. coli* occurred only in children aged less than 5 years (Agbonlahor et al., 1982).

3. **Salmonella**

Some 2000 serotypes of *Salmonella* are known, any of which, with the possible exception of the *S. typhi* can cause acute diarrhoeal disease. But the large majority of outbreaks are caused by *S. typhimurium*, *S. enteritidis* and about a dozen other serotypes.

*Salmonella* invade the ideal epithelial cells, penetrate to the lamina propria and usually initiate bacteriemia, with fever and other systemic manifestations. An enterotoxin has also been demonstrated in some serotypes, such as *S. typhimurium*. The pathogenic significance of the enterotoxin is not known.
*Salmonella* frequently harbour plasmids, which confer resistance to multiple antibodies.

A case control study in 5 food net sites to identify risk factors for sporadic infant salmonellosis. A case patient was a child under 12 months of age with a laboratory confirmed, non typhoidal serogroup B or D *Salmonella* infection. 22 case patients were matched with 39 control subjects. In a multivariate analysis, case patients were more likely to have a liquid diet containing no breast milk than a liquid diet containing only breast milk. To decrease the risk of salmonellosis, mothers should be encouraged to breast feed their infants (Rowe *et al.*, 2004).

A retrospective study was conducted on 41 patients between 1994 and 2002 in Casablanca University hospital, Paediatric ward, Morocco. Non-typhoidal salmonellosis (NTS) infections are a major cause of infantile death in developing countries. 20 cases of digestive Salmonellosis were diagnosed. 10 patients less than 3 months of age were hospitalised after an outbreak of resistant *Salmonella typhimurium* in a nursery. Fever, vomiting and diarrhoea was noted in 97% of cases. 55% of cases had severe dehydration. *Salmonella* strains were identified in blood of 25 cases. The following *Salmonella* serotypes were identified: *S. typhimurium* (53.6%), *S. enteritidis* (44%) and *S. agona* (2.4%) and *Salmonella typhimurium* (34%) showed resistance to antibodies (Ailal *et al.*, 2004).

4. **Shigella**

All four serogroups of *Shigella* produce dysentry. The pathogenesis of dysentry depends on the penetration of epithelial cells of the large bowel and subsequent local multiplication, producing colitis. In addition, *Shigella* may also produce a mild watery diarrhoea. This is due to the action of an exterotoxin on the same bowel.
Shigellosis is worldwide, being more common in poor, overcrowded and unhygienic communities. It has been described as a water washed disease, its incidence decreasing as the amount of water used for sanitation increases. *S. flexneri* is the commonest type in the developing countries and *S. sonnei* in the developed countries. *S. dysentriae* type 1 causes the most serious types of dysentery.

Sultana *et al.* (1981) in their study in Bangalore, India stated that diarrhoea was high among children aged under 2. *Shigella* was isolated from 27 cases. *Shigella dysentriae* (18) was commonest followed by *Shigella flexneri* (5) and *Shigella sonnei* (4).

The association of breastfeeding with the risk of microbiologically confirmed or clinically presumptive Shigellosis was assessed in a case control study of Bangladeshi children younger than 3 years. 269 cases with culture confirmed Shigellosis (n=119) or clinically presumptive shigellosis(n=150) were compared. The ratio relating breastfeeding to confirmed or presumptive Shigellosis suggested a substantial protective effect. Breastfeeding is a central component of Shigella control programs in less developed settings. Breastfeeding was associated with 52% reduction in risk (Ahmed *et al.*, 1992).

5. *Clostridium perfringens*

*C. perfringens* (C. welchii) is a spore forming bacillus widely distributed in soil and frequency found in the intestinal tract of man and animals. Five types (A to E) are recognised. Most cases of human clostridial gastroenteritis are caused by Type A. Certain heat resistant non-hemolytic strains of Type A have been recognised as important cause of food poisoning. Various heat sensitive hemolytic strains have also been reported to cause diarrhoea.
In contrast to the non fatal water diarrhoea caused by type A strains sometimes C strains can cause a severe hemorrhagic necrotizing jejunitis leading to bloody diarrhoea, severe abdominal pain and shock. Type A strains produce an enterotoxin which appears to be a structural component of the spore coat. Type C strains also produce a related enterotoxin.

Persistent diarrhoea was an important problem among children during the first 2 years of life. There were no significant sex-related differences in the incidence of the disease, and the overall seasonal distribution of acute and persistent diarrhoea was similar. In order to determine the descriptive epidemiology of persistent diarrhoea in rural northern India, a cohort of 963 children aged 0-71 months was followed prospectively for 12 months through weekly household visits. The incidence of persistent diarrhoea and passage of gross blood with stools (P less than 0.001) was 6.3 per 100 child-years among those aged 0-71 months, and was highest (31 per 100 child-years) among those aged 0-11 months. The persistence of diarrhoeal symptoms was significantly correlated with a higher initial mean stool frequency (P less than 0.01). Established enteric pathogens were isolated during the initial illness in 46.4% of persistent and 55.4% of acute episodes. Pathogens isolated during persistent episodes included enterotoxigenic Escherichia coli (ETEC 9.3%), Salmonella spp. (4.7%), as well as Campylobacter (4.7%), Shigella spp. (2.3%), Entamoeba histolytica (2.3%), and Rotavirus (2.3%). Similar proportions of these pathogens were isolated also during episodes of acute diarrhoea. Multiple pathogens were isolated in 7% of the persistent and 5% of the acute episodes. E. coli that manifested aggregative adherence (EAEC-A) was more common (34.9% versus 12.3%) in persistent than acute episodes (P less than 0.01), and initial faecal excretion of EAEC-A was significantly associated with the persistence of a diarrhoeal episode (Bhan et al., 1989).
2.10 AETIOLOGY OF GASTROENTERITIS

Bacillus cereus: This is a ubiquitous aerobic spore bearing bacillus. Ingestion of food heavily contaminated with *B. cereus* leads to diarrhoea. Two clinical syndromes are caused by this bacillus, one characterised by diarrhoea and abdominal cramps and the other by nausea and vomiting, resembling staphylococcal food poisoning. Both types appear to be caused by enterotoxin.

Staphylococcus aureus: Some strains of *Staphylococcus* produce enterotoxin. When food gets contaminated with such strains, they multiply and elaborate the toxin, which is heat stable. Digestion of such food results in vomiting and diarrhoea.

2.11 COMMENSAL MICROBIOTA OF THE GASTROINTESTINAL TRACT

The foetal intestine is sterile and bathed in swallowed amniotic fluid. Following delivery, the colonisation of the intestines by a variety of microorganisms begins. Gastrointestinal colonisation involves a succession of bacterial populations, waxing and waning as the diet changes and the host develops. The assemblage of bacteria inhabiting the gut is usually referred to as the commensal intestinal microbiota. The colonic microbiota is dominated by obligatory anaerobes such as *Bacteriodes spp, Clostridium spp, Bifidobacteria, Eubacteria and Fusobacteria*. Bacteria may be free living in the lumen or attached to the mucus, mucosa surface, food particles or digestive residues. Factors influencing the intestinal microbiota composition can be divided into host factors (such as pH, transit time, bile acids, pancreatic enzymes and the mucus composition), non host factors such as adhesion capacity, enzymes and metabolic capacities. The bacteria can infer important health benefits for the human host. The most important
function of the gut microbiota is colonisation resistance. By competing for nutrients and adhesion sites, but also by the production of antibacterial substances (bacteriocins), the indigenous gut microbiota makes it difficult for entry of potentially pathogenic bacteria to colonize. Gut microbiota plays an important role in the development of the gastrointestinal immune system. Major changes in the intestinal microbial composition occur especially early in life.

Generally the intestinal microbiota of breast fed infant are primarily lactic acid bacteria, like *Bifidobacteria* and *Lactobacilli*. The microbiota of bottle fed infants, on the other hand, are more diverse, generally *Clostridium* and Enterobacteriaceae.

The new born infant is more susceptible to infections than older children or adults. This is because all three lines of defense against microbial invasion (i.e.) barrier functions, innate immunity and specific immunity.

Diarrhoea is the leading cause of illness and death among children in developing countries where an estimated 1.3 thousand million episodes and 3.2 million deaths occur each year in those under five years of age. About 80% of deaths due to diarrhoea occur in the first two years of life.

The colonization of the new-born gastrointestinal tract by bacteria is a complex process which implicates competition for oxygen, nutrients and ecological niches and is strongly influenced by infant diet breast or formula feeding.

The main cause of death from acute diarrhoea is dehydration, which results from loss of fluid and electrolytes in diarrhoeal stools.
2.12 TYPES OF DIARRHOEA

In epidemiological studies diarrhoea is defined as the passage of three or more loose or watering stools in a 24 hr. Infants who are exclusively breast-fed normally pass several soft or semi-liquid stool each day; for them, it is practical to define diarrhoea as an increase in stool frequency a liquidity that is considered abnormal by the mother. Three clinical syndromes of diarrhoea have been defined, each reflecting a different pathogenesis and requiring different treatments.

**Acute watery Diarrhoea**

This type of diarrhoea begins acutely, lasts less than 14 days and involves the passage of frequent loose or watery stools without blood. This causes dehydration and contributes to malnutrition.

Causes: Acute watery diarrhoea in young children in developing countries are mainly due to rota virus enterotoxigenic *E.coli, shigella, Campylobacter jejuni and Cryptosporidium.*

**Persistent Diarrhoea**

This diarrhoea begins acutely but is of unusually long duration. The episode may begin as watery diarrhoea. Marked weight loss is frequent. Diarrhoeal stool volume may be also be great with the risk of dehydration.

Causes: Enteroaggregative *E.coli, Shigella and Crypto sporidium*

**Chronic Diarrhoea**

This is recurrent or long lasting diarrhoea.
Causes: This type of diarrhoea is caused by sensitivity to gluten, inherited metabolic disorders.

Mechanism of Watery Diarrhoea

There are 2 principal mechanisms by which watery diarrhoea occurs.

1. Secretion
2. Osmotic action

Secretary Diarrhoea

Secretary diarrhoea is caused by the abnormal secretion of water and electrolytes into the small bowel. This occurs when the absorption of sodium by the villi is impaired while the secretion of chloride in the crypt cell continues or is increased. The net result is fluid secretion which leads to the loss of water and salts from the body as watery stools; this causes dehydration.

In infectious diarrhoea, these changes may result from the action of the bacterial toxins on the bowel mucosa such as those of Escherchia coli and Vibrio cholerae 01.

Osmotic diarrhoea

The small bowel mucosa is a porous epithelium across which water and electrolytes move rapidly to maintain osmotic balance between the bowel content and the ECF. Under these conditions, diarrhoea can occur when a poorly absorbed, osmotically active substance is ingested. If the substance is taken as an isotonic solution, the water and the solute will simply pass through the gut unabsorbed, causing diarrhoea. Purgatives such as magnesium sulphate work by this principle.
*E. coli* can cause diarrhoea by a variety of mechanisms. These include release of either heat labile or heat stable enterotoxin and invasion of the bowel mucosa. Recently, a third mechanism has been proposed, the ability of the bacteria to colonise and adhere to the small intestinal mucosa (Ulshen and Rolls, 1980).

### 2.13 PRE-DISPOSING FACTORS TO GASTROENTERITIS

Various explanations have been put forward to account the resistance of breast fed babies to gastroenteritis. These include the passive transfer of antibodies to EPEC in colostrum (Sissman, 1961), contamination of artificial feed during preparation. (Nater & Buffalo, 1959) and the nature of intestinal environment (Ross & Dawes, 1954). In their detailed and excellent study of the problem, Ross and Dawes concluded that the preponderance of lactobacilli in the faeces of the breast fed infants and the relatively low pH of the large bowel contents were the main factors responsible for the natural resistance to enteric infection. They found that the oral feed of lactose had only a partial and temporary effect in reducing the pH of faeces of artificially fed babies and they suggested that human milk must contain another factor necessary for the maintenance of an acid pH and a lactobacillary flora.

In India, 1.4 million children die annually from diarrhoeal diseases other than cholera (Ananthanarayanan and Panicker, 1999). Apart from causing death due to dehydration, diarrhoeal diseases often initiate malnutrition, which is aggravated by each successive episode.

343 infants were studied in a hospital based descriptive study to assess the relationship between breastfeeding practices and morbidity from respiratory and diarrhoeal diseases in infants. 3 broad groups-exclusively breastfed for 3 months or less, 4 months or more, and those who were never breastfed. Not affected was the
second group, least affected was the first group and those who were never breastfed showed worst results. This study once again proved the extended protective effect of exclusive breastfeeding for periods of over 4 months against respiratory and diarrhoeal diseases (Perera et al., 1999).

A survey conducted by the programme for control of diarrhoeal diseases (WHO 1983) has indicated that in 1980 close to 1 billion episodes of acute diarrhoea occur in children under five years of age in under developed and developing countries. It is estimated that five million children under five years of age died in 1980 from intractable diarrhoea. At this rate two young children die from chronic diarrhoea every minute of the day, an abstract figure that explains both the magnitude and devastation of its effect (Labenthal, 1984).

A case control study of the relationship between feeding mode and risk of hospitalised diarrhoea in infants (aged 2-11 months) in Basrah city was conducted from September 1983 to May 1984. A total of 597 cases were selected among infants admitted with diarrhoea to the major paediatric hospital in the city. For infants aged 2-3 months, breast feeding alone or breast feeding plus food were the least risky feeding modes. Bottle feeding alone was associated with diarrhoea (Mahmood et al., 1989).

In a prospective study on breast feeding in Jerusalem, significant differences in the number of episodes of illness were found between breast fed and bottle fed infants at 20 weeks; infants exclusively breast fed had the least number of illness episodes. The duration of breast feeding and the number of illness episodes had a positive association. 52% had no episode compared with only 15% who were not breast fed (Palti et al., 1984).
A case control study in Brazil has shown that young infants who are not breast fed have a 25 time greater risk of dying of diarrhoea than those who are exclusively breast fed. Breast feeding helps maintain hydration status during diarrhoeal episodes. A longitudinal study in the urban slums of Lima, Peru found that exclusively breast fed infants have a reduced risk of diarrhoeal morbidity. These studies and other factors in developing countries point to the need to extend the duration of exclusive breastfeeding for at least 4-6 months. The addition of supplementary foods early in infancy under prevailing environmental conditions in developing countries leads to their increased diarrhoeal attacks and associated reduced food intake. Thus breastfeeding is important in providing necessary calories and protein during a time when the infants often refuse foods is common (Huffman et al., 1990).

In a study conducted in Kuwait among 204 infants of whom 59(29%) were breastfed, 115(56%) artificially fed and 30(15%) were on mixed feeding (breast and artificial) at the time of admission. 45(22%) were aged 1-3 months. The results of this study suggest that many factors other than breast feeding are associated with frequency of infant admission for acute diarrhoea. The highest rate of re-admission was among infants on bottle feeding who received unfiltered water and infants on breast feeding who received mineral water, the lowest rate of readmission was among infants on breast feeding who received boiled water. Breast milk is the best infant food where water supplies are bad and hygiene is difficult (Portobian et al., 1995).

Acute diarrhoea in Sub-Saharan countries can be caused by broad spectrum of viral, parasitic and bacterial enteropathogens. The contribution of the various pathogens to diarrhoea may differ substantially depending on local, geographic and socio-economic conditions. Bacterial enteropathogens were detected more frequently in patients. Shigella boydii, Shigella flexneri and Salmonella enterica
were isolated. PCR identification of enteropathogenic *Escherichia coli* was done (Klaus Reither Ralf *et al.*, 2007).

Death among children younger than 5 years in low income countries is caused by diarrhoeal diseases. GEMS-a 3 year prospective, age, stratified, matched case control study of moderate to severe diarrhoea in children aged 0-59 months residing at four sites in Africa and three in Asia. 9439 children with moderate to severe diarrhoea were analysed. Bacterial pathogens identified were Enterotoxigenic *Escherichia coli* producing heat stable toxin (ST-ETEC) and *Shigella* (Kotloff *et al.*, 2013).

A historical cohort study of the associations between diarrhoea and feeding modes and severity in children aged 0-14 months at baseline was done in Al Ain City, UAE. A three months follow up of 249 children revealed that non breastfed had more diarrhoea than did partly breastfed. The protective effect of breastfeeding against infantile diarrhoea may be less pronounced in areas with modern water supply and sanitation facilities. Significant differences were seen only between non breastfed and fully breastfed subgroups. Breastfeeding plays an important role in reducing the incidence and severity of infantile diarrhoea (al-Ali *et al.*, 1997).

WHO and CDD is looking at ways to prevent diarrhoea and has identified breastfeeding as an important factor. CDD has identified trials of interventions for breastfeeding promotion and the evaluation of approaches. CDD also has included breastfeeding promotion. The programme plans to monitor the effectiveness of the training and develop future based on that feedback.
2.14 **RISKS OF FORMULA FEEDING FOR INFANTS**

The effects of formula feeding an infants and children continue to be studied and several significant risks have been identified. There is increased risk of mortality - this applies to western countries and developing countries.

2.15 **DISADVANTAGES OF BOTTLE FEEDING**

Despite the advanced formulas available on the market today, formula milk do not quite match the nutrients and antibodies that breastfeeding can provide. Bottles need to be constantly cleaned and for the first 6 months, the water needs to be boiled and then allowed to cool.

2482 children aged 0-23 months from Bangladesh were studied. Poor infant and young child feeding practices are contributing to the burden of infectious diseases and malnutrition. 27.5% of mothers initiated breastfeeding within the first hour after birth, 97.3% were currently breastfeeding, 99.9% had ever breastfed their infants and 222.4% were currently bottle feeding. Infants under 6 months of age, 42.5% were exclusively breastfed, 62.3% received complimentary foods in addition to breast milk. Risk factors for an infant not being exclusively breastfed were higher socio-economic status (Mihrshani *et al.*, 2010).

Infant formula use as standard practice, supportive perceptions of formula feeding as normative and hindering translation of current research into supporting exclusive breastfeeding. In this review the author analysis literature on exclusive breastfeeding and converted reported odds to allow discussion of the risk of formula use. Exclusive breastfeeding is an optimal practice, compared to other infant feeding practices that carry risks (Mc Niel *et al.*, 2010).
In their study evaluated the protective role of breastfeeding against diarrhoea in children younger than one year of age in the city of Feira de Santana, Brazil in 2001. Out of the 2319 children evaluated, 11.6% had diarrhoea with greater frequency after the sixth month (63.3%). Diarrhoea was 64% higher in children younger than 6 months who were bottle fed vs breastfed children. When compared to the children who were exclusively breastfed, the chance of diarrhoea increased to 82% in children who were not breastfed (95%). Breastfeeding and exclusive breastfeeding were a protective factor against diarrhoea in the first six months of life (Vieira et al., 2003).

133 infants less than 1 year of age in a poor urban settlement in Brasilia were studied. Osmolity of breast milk was significantly less than that of bottle milk. The wide range of osmotic strength found in bottle milk was partly due to the predominant use of (82%) powdered cow’s milk among non-breast feeders and its improper reconstitution (Dorea et al., 1988).

A household survey of diarrhoea among 4458 children under age of 5 years in Dakahlia, Egypt from June 2002 to May 2003 was conducted to determine the prevalence and determinants of diarrhoeal disease. Diarrhoeal frequency in last 24 hours and previous two weeks were 23.6% and 8.75 respectively. ORS use rate was 24.3%. The frequency was higher in rural areas, those aged 6-24 month young mothers, lower education also were deciding factors (El-Gilany, 2005).

Two districts of Haryana State were selected and a community based study to assess the problem of diarrhoea amongst children below 3 years of age. 818 children in the age group of 0-35 months. The occurrence of diarrhoea was highest in the age group 6-11 months (28%) and was lowest in age group 0-5 months (16%). 37% of children suffering from diarrhoea received bottle feed (Kapil et al., 1994).
1926 children aged less than 5 years were studied in Goroka town, Lowa and Asaro census divisions between 1986 and 1989 for morbidity due to diarrhoea. The average number of episodes was three. Incidence of diarrhoea was highest in the remote Asaro census division. Incidence of diarrhoea was higher in January than at other times of the year. It is necessary to improve access to water, improve hygiene practices and breastfeeding to reduce diarrhoea morbidity (Wyrsch et al., 1998).

A case control study was conducted in the metropolitan area of Porto Allegre, Brazil on 192 children 0-23 months hospitalised with diarrhoea with moderate to severe dehydration. The associations between dehydrating diarrhoea and type of milk consumed, time since breastfeeding stopped and breastfeeding status were measured. The risk of dehydrating diarrhoea was higher in first 9 months of life. The type of milk given before diarrhoea started had a strong association with dehydration independent of socio economic, environmental, demographic and health service factors. Bottle fed infants were at greater risk than exclusively breastfed infants for cow's milk and formula milk. Children who stopped breastfeeding in the previous 2 months were more likely to develop diarrhoea when compared to those still breastfeeding. These results confirmed the protective effect of breastfeeding and there is a susceptible period soon after breastfeeding is stopped (Fuchs et al., 1990).

In India, poor feeding practices in early childhood contribute to infant and child mortality. A national Family Health Survey 2005-2006, targeted interventions may be necessary to improve infant feeding practices in mothers who reside in urban areas (Patel et al., 2010).

Fourteen municipalities of greater Sao Paulo were studied. The fractions of mortality preventable by breastfeeding were calculated. The variation for
diarrhoea was 35% and 86%. The impact of breastfeeding over infant mortality was an average reduction of 9.3% with values ranging from 3.6% to 13% depending on the locality considered. Breastfeeding in the first year of life is the most feasible strategy to reduce the current level of infant mortality in the state of Sao Paulo (Escuder et al., 2003).

In a study of 170 healthy newborns, incidence and duration of acute respiratory infection and diarrhoea were recorded biweekly, Infants were classified as fully or breastfed or formula fed. ARI was negatively associated with duration of breastfeeding. Infants that were never breast fed were more likely to have an episode of ARI than those fully breastfed for at least 1 month. (Lopez Alarcon et al., 1997). In a cross-sectional survey conducted in the rural district of Hoima, Western Uganda of 720 child/mother pairs concluded that breastfeeding was universal (99%), prelacteal use was high (43%). Median duration of exclusive breastfeeding was 3.5 months. 10% were bottle fed, 92% were breast fed, 21% received complementary food. 36% of breastfeeding children received dairy milk (Wamani et al., 2005).

2.16 ORAL REHYDRATION THERAPY

ORT was introduced in the early 1980's the 25th anniversary of the discovery of oral rehydration therapy (ORT), the corner store of diarrhoea control efforts (Classon and Merson, 1990) was commemorated in 1994. This breakthrough has been described as 'potentially the most important medical advance this century has witnessed. Nevertheless, there is limited evidence that ORT has been able to reduce diarrhoea mortality on a large scale.

Oral rehydration solutions were developed in the 1940"s and the effect of potassium replacement in reducing mortality was recognised, which led to
substantial decrease in case fatality rates. Effectiveness of IV rehydration fluids among economically disadvantaged populations provided an impetus to develop less expensive but equally effective oral solutions. Studies published in 1968 from Dhaka and Calcutta demonstrated the effectiveness of ORS for cholera patients, including those with high stool output. The resulting success of oral solutions fastened the development of the first world health organisation (WHO) ORT and the production of standard packets of oral rehydration salts. Now, ORT is accepted as the standard of care for the clinical efficacy and cost effective management of acute gastroenteritis. During diarrhoeal disease, volume of intestinal fluid output is increased, overwhelming the re-absorptive capacity of the gastrointestinal tract. Treatment with ORS is simple and enables management of uncomplicated cases of diarrhoea at home regardless of etiologic agent. All families should be encouraged to have a supply of ORS at home at all times. Infants with acute diarrhoea are more prone to becoming dehydrated than are older children because they have a higher body surface to volume ratio, a higher metabolic rate, smaller fluid reserves and they are dependent on others for fluid. Parents of infants with diarrhoea should seek medical evaluation as soon as the child appears to be in distress. No guidelines have established a specific age under which evaluation is mandated.

In a 4 year study in the hospital Infantil del Estado de Sonoro, children less than one year received oral rehydration therapy in 86.8% cases. Effectivity of ORT was in 90.9%. 92.8% in light dehydration and 787% moderate. Reduction in hospitalisation was 19.2% in 1986 and 38.4% in 1989. Diarrhoeal mortality decreased in urgency department in 42% and in the infectology department in 54% (Dohi-Fujii et al., 1993).

In a ecological study in Ceara, North east Brazil, the variations in diarrhoea specific infant mortality were examined. Diarrhoea is the main cause of post-natal
death in Ceara. Interrelationship between diarrhoea-specific infant mortality were predicted. The significant determinants of variability in diarrhoea specific infant mortality was exclusive breastfeeding. The findings suggest that community based promotion of exclusive breastfeeding in the first 4 months reduce mortality (De Souza, et al., 2001).

In Egypt, there was an acceleration in the rate of fall in diarrhoea, mortality after a rational ORT campaign started in 1983 (El.Rajie et al., 1990). In rural Bangladesh however a massive ORT campaign failed to reduce overall diarrhoea mortality (Fanvean, 1992).

Case control study of diarrhoeal disease cases presenting to 34 general practices in England revealed data on 304 infants aged 6 months. The effect of receiving no breast milk was stronger in more deprived areas than in less deprived areas. In formula fed infants, there was significantly more diarrhoeal disease in those not sterilising bottles/teats with steam or chemicals. The protective effect of breast feeding did not persist beyond 2 months after breast feeding was stopped. Breast feeding protects against diarrhoeal disease in infants in England although the degree of protection may vary across infants and wear off after breast feeding and the risks of inadequate sterilisation should be targeted in deprived areas or households (Quigley et al., 1991).

2.17 EFFECT OF FEEDING ON DIARRHOEA

The notion that feeding should be reduced or stopped during diarrhoea reflects a common belief that giving food will cause stool output to increase and thus make the diarrhoea worse, but this is not usually well tolerated during diarrhoea. Children who continue to breast feed during diarrhoea actually have
reduced stool output and a shorter duration of illness than children who do not breast feed.

A cross-sectional study of 771 children was conducted in urban Ilorin, Nigeria to determine socio-economic conditions and feeding practices relation to diarrhoeal disease among infants. The lowest diarrhoeal rate occurred in infants aged 0-3 months while highest rate occurred among infants 7-9 months old. Diarrhoeal disease awareness campaign to educate mothers on the dangers of childhood diarrhoea and how to prevent it through proper hygiene is advocated (Oni, 1996).

Children on animal milk or formula milk may have some increase in stool volume.

2.18 SORBITOL MACONKEY AGAR

March and Ratnam (1989) reported that screening of stools for *E.coli* 0157; H7 used Sorbitol MacConkey agar and checked colonics typical of *E.coli* for sorbitol fermentation. After sorbitol negative colonies were detected, the next step performed was a test to detect the 0157 antigen. Biochemical tests were also done to confirm the isolates as *E.coli*. 29% performed a test to detect the 0157 antigen and 48% performed biochemical tests to confirm to isolates as *E.coli*, 43% performed both 0157 antigen testing and biochemical tests to confirm the isolates as *E.coli*.

The recommended method centre for Disease control and prevention 1994) for screening *E.coli* 0157:H7 is relatively simple and is based on the fact that *E.coli* 0157:H7 does not ferment sorbitol rapidly (March and Ratnam., 1986). Stool is plated on sorbitol MacConkey agar, which contains 1% sorbitol instead of lactose. After 18 to 24 hrs of incubation, organisms that ferment sorbitol appear as
pink colonies. Non-sorbitol fermenting organisms, including *E.coli* 0157 : H7 are colourless. If these are no sorbitol negative colonies, the specimen needs no further testing.

If the Sorbitol MacConkey plate contains sorbitol negative colonies, these are selected and assayed for the 0157 antigen with commercially available antiserum (March and Ratnam 1989), because of possible cross-reactions with other organisms, sorbitol negative or organisms that agglutinate in 0157 antisera should then be biochemically identified as *E.coli*. Performing the 0157 antigen testing before biochemical identification will save time, because antigen testing is the quicker procedure; isolates testing negative for the 0157 antigen need not be biochemically identified as *E.coli*.

So identification and confirmation should be done by biochemical testing, antigenic typing and toxin testing.

2.19 TYPES OF *E.coli*

There are four major categories of diarrhoeagenic *E.coli*, enterotoxigenic (a major cause of traveller's diarrhoea and infant diarrhoea in less developed countries) enteroinvasive (a cause of dysentery), entero pathogenic (an important cause of infant diarrhoea) and entero hemorrhagic (a cause of haemorrhagic colitis and hemolytic uremic syndrome) - Besides manifesting distinct clinical patterns, these categories of *E.coli* differ in their epidemiology and pathogenesis and in their O : H serotypes. Common features include plasmid encoded properties, characteristic interactions with intestinal mucosa and elaboration of various types of enterotoxin or cytotoxin. Organisms in these categories differ in their pathogenesis, clinical features, epidemiology and O : H serotypes (Lesine,
ETEC strains have been characterised by their ability to produce heat labile (LT), heat stable (ST) or both enterotoxins (Lesine, 1987). For diarrhoea to result, ETEC must first colonize the small bowel epithelium by means of fimbriae on its cell surface, also called colonization factor antigens (CFA). So far, three major CFA have been described.

ETEC isolated from infants with diarrhoea in Chile was a preliminary step in determining the enteropathogenic role of ETEC. This characterization was achieved by standard biological assays and by DNA-DNA hybridization analysis (Guerrant et al., 1986).

Among children in developing countries, Enterotoxigenic Escherichia coli (ETEC) alone is responsible for more than 650 million diarrhoeal episodes and 2,000,000 deaths annually. However, in most developing countries including Kenya, there is a paucity of data on prevalence and characteristics of specific bacterial etiology. Antibiotic susceptibility tests were performed on all isolates using the disc diffusion and minimum inhibitory concentration methods. ETECs as well as other Enteric bacteria were resistant to more than four antibiotics (Chloramphenical (28%), Cotrimoxazole (78%), Co-amoxillin (70%) Erythromycin (98%) Ciprofloxacin (5%), Cefotaxime (18%) and Tetracyclin (56%). Those found resistant to third generation cephalosporins were tested for Extended Spectrum Beta Lactamases (ESBL) while those resistant to quinolones were tested for Gyrase resistance genes (Gyrase A, B and Topoisomerases). Sequencing was done on all Gyrase and topoisomerase positive isolates for detecogies of diarrhoeal illness.
Antibody titres against *Escherichia coli* in human milk and in sera of 11 breastfed infants, 6 bottle fed infants and 9 infants in post weaning period were measured by passive haemagglutination method. High antibody titres were seen in breast milk in the first 4 days. No healthy breastfed infants had serum antibodies, while a breastfed infant with *E.coli* abscess had antibodies. 4 of the 6 bottle fed infants and all the 9 infants in the post weaning had antibodies (Ogura, 1987).

ETEC was detected significantly more often in children less than five years old (25/300). A high incidence of *E.coli* isolation (72.3%) was found among 100 households in low income group in Chandigarh. Most of it was from feeding bottle nipples. 80 out of the 100 households had history of infantile diarrhoea exhibiting 80.9% *E.coli* isolation. 79% of storage containers of water exhibited the presence of *E.coli* (Ghuliani et al., 1995).

### 2.20 EPEC

Enteropathogenic E.coli (EPEC) has been implicated as a cause of diarrhoea and gastroenteritis in infants (Tewari et al., 1979). Prevalence of EPEC in infants (0-24 months) with diarrhoea was studied in National Hospital, Abuja, Nigeria and EPEC is one of the most important etiologic agents of infantile diarrhoea in developing countries (Kandakai et al., 2008).

WHO international Escherichia Centre listed 150 subgroups of *E.coli* as entero pathogenic to man (Thoren, 1983). Sakazaki et al. (1967 and 1974) identified OH groups of *E.coli* as possible enteropathogens. Experiments with animal models and human subjects have shown that EPEC can cause ‘intestinal secretion and diarrhoea’ (Thoren, 1983).

The mechanism of production of diarrhoea by EPEC may be the production of an enterotoxin which is different from those produced by enterotoxigenic *E.coli*
(Klipstein, 1978) or a mechanism related to adhesion and colonization of the small intestine (Williams et al., 1978; Craviota et al., 1979 and Darferille et al., 1983).

EPEC is an important cause of infantile epidemic diarrhoea and is a likely cause of endemic diarrhoea (Bray, 1945 and Neter, 1959). Workers in Western Countries have isolated EPEC in 7% to 30% diarrhoeal cases (Hugh et al., 1968; Gurwith and Williams 1977; Edelnan and Levin, 1983). Studies from India detected EPEC in 8% to 24% of diarrhoeal cases in children (Prakash, 1962, Ganguly et al., 1980, Kanwar et al., 1983 and Sen et al., 1983).

EPEC exhibit localised adherence to Hep 2 cells, whereas class II EPEC exhibit either diffuse adherence or no adherence to all Hep 2 cells. The present study was to investigate the relative contribution of enteropathogenic Escherichia coli (EPEC) as a cause of infectious diarrhoea in Norwegian children. The EPEC category can be conveniently subdivided into two classes on a rational pathogenic basis. These include strains referred to by some authors as attaching and effacing E.coli (AECC) (Moon et al., 1983). Class I There is also a fifth category of diarrhoeagenic E.coli, enteroadherent E.coli (EAEC) (Mathewson et al., 1985). Although little is known about the pathogenesis, therefore they have been related to a fifth category (Lerine, 1987). The aim of Data from faecal specimens from children <2 years old with diarrhoea during the year 2001 were analysed. E. coli isolates with the attaching and effacing genotype (eae+) were examined for the presence of the bundle-forming pilus (bfpA) and Shiga toxin genes by PCR and for genetic relatedness by PFGE. During the 1-year period, 598 specimens from 440 patients <2 years old were analysed. Epidemiology and serotypes of the strains in this category of E.coli, preliminary evidence suggests that they are indeed capable of causing diarrhoeal disease and they clearly do not fit into the other four categories, t Potential enteric pathogens were identified in 124 patients
EPEC was the most frequently identified agent (44 patients), followed by rotavirus (41 patients), *Campylobacter jejuni* (17 patients) and adenovirus (17 patients). All other agents were detected in five patients or less. Only one of the *eae*+ *E. coli* isolates was classified as typical EPEC (bfpA+). Among the 43 isolates that were classified as atypical EPEC (bfpA-), eight strains belonged to EPEC serogroups, whereas the majority of strains (n = 35) were not agglutinated by EPEC antisera. None of the EPEC isolates were genetically related. This study demonstrates that atypical EPEC of non-EPEC serogroups is highly prevalent among Norwegian children with diarrhoea.

More than 1 billion episodes of diarrhoea occur among children younger than 5 years of age every year in socio economically developing countries causing 2 to 5 million deaths. *Escherichia coli* are the most common pathogens responsible for acute diarrhoea episodes in children. *Shigella* spp, *Salmonella* spp, *Vibrio cholerae* occur in poorer areas commonly. Major therapeutic intervention for all individuals with diarrhoea consists of fluid and electrolyte therapy. When antimicrobial therapy is appropriate, selection of a specific agent can be made based upon susceptibility patterns of the pathogens or information on local susceptibility patterns. Preventive measures include careful personal hygiene and frequent hand washing (O'Reyan *et al.*, 2005).

In their study at the Dhaka hospital of infants (0-11 months), the prevalence of breast feeding in infants attending the hospital with diarrhoea reduced from 31% in 2008 to 17% in 2011, with corresponding increase in prevalence of non-breastfed. The protective role of breast feeding in infantile diarrhoea caused by major viral and common bacterial agents. These findings emphasise the importance of promotion of breast feeding in Bangladesh and elsewhere (Dev *et al.*, 2013).
168 cases of ETEC diarrhoea among Bangladesh infants and children <36 months of age were studied. When compared with other feeding modes, exclusive breastfeeding of infants was associated with significant protection against severe ETEC diarrhoea (Clemens et al., 1997).

### 2.21 ANTIBIOTIC SENSITIVITY TESTING

Pattern of bacterial sensitivity and resistance vary considerably from place to place. Hospitals or even wards often have their non particular resistance problems.

Therapy of infection normally begins, quite properly, before laboratory results are available. Antibiotic sensitivity testing primarily plays a supplementary role in confirming that the organism is susceptible to the agent that is being used. Sometimes it may enable the clinician to change from a toxic to a less toxic agent or from an expensive to a cheaper one.

Sensitivity testing is used to establish the degree and spectrum of in vitro activity of new antibacterial agents, first of all in the laboratories of the pharmaceutical houses where most new developments emanate and also in diagnostic laboratories where the new agent can be tested against the various types of organism encountered locally.

In a study conducted in Igembe, Kenya on 308 children ETEC 9.1%, EPEC 6.8% and EAEC 12.3%, *S. paratyphi* 10.4%, *Shigella flexneri* 1.9% and *Shigella dysenteriae* .9% were isolated. 95% of the isolates were resistant to Amoxicillin and Cotrimoxazole (Shirley et al., 2002).

In developing countries, diarrhoeal diseases are one of the major causes of death in children under 5 years of age. It is known that diarrhoeagenic *Escherichia*
coli (DEC) is an important aetiological agent of infantile diarrhoea in Nicaragua.

The study was to determine the antimicrobial resistance pattern in a collection of 727 intestinal E. coli isolates from the faeces of children in León, Nicaragua, between March 2005 and September 2006. All samples had been screened previously for the presence of DEC by multiplex PCR. Three hundred and ninety-five non-DEC isolates (270 from children with diarrhoea and 125 from children without diarrhoea) and 332 DEC isolates (241 from children with diarrhoea and 91 from children without diarrhoea) were analysed in this study. In general, antimicrobial resistance among the 727 intestinal E. coli isolates was high for ampicillin (60%), trimethoprim-sulfamethoxazole (64%) and chloramphenicol (11%). Among individual E. coli categories, enteroaggregative E. coli isolates from children with and without diarrhoea exhibited significantly higher levels of resistance (P<0.05) to ampicillin and trimethoprim-sulfamethoxazole compared to the other E. coli categories. Resistance to ceftazidime and/or ceftriaxone and a pattern of multi-resistance was related to CTX-M-5- or CTX-M-15-producing E. coli isolates. The results suggest that E. coli isolates from Nicaraguan children have not reached the high levels of resistance to the most common antibiotics used for diarrhoea treatment as in other countries (Amaya et al.,2011).

Diarrhoea is one of the main causes of morbidity and mortality among children in sub-Saharan Africa, and one of the main causes of hospital admissions in rural areas of Kenya. In Kenya, antimicrobial resistance surveillance has been conducted only at the institutional levels, with limited sharing of information and analysis of data. As a result, the actual scale of regional or national antimicrobial drug resistance is not well defined.

Stool samples were collected between 1 October 2007 and 30 September 2008 from a total of 651 outpatients with diarrhoea who were under five years of age in four provinces of Kenya. Conventional, biochemical methods, multiplex
PCR and antimicrobial susceptibility were conducted to identify the bacterial causes and virulence factors in the isolates, respectively.

Of the 651 patients screened, identified the causes of 115 cases (17.7%) as follows: Pathogenic E. coli (11.2%) [enteroaggregative (8.9%), enterotoxigenic (1.2%), enteroinvasive (0.6%), shigatoxigenic (0.5%)], Salmonella (3.5%), Shigella (2%) and Vibrio cholera O1 (0.7%). The highest levels of resistance among the E. coli isolates were observed in ampicillin and trimethoprim/sulphamethoxazole each at 95% followed by tetracycline at 81%. Shigella isolate levels of resistance ranged from 80% to 100% for ampicillin, tetracycline and trimethoprim/sulphamethoxazole. The highest prevalence of antimicrobial resistance was to ampicillin followed by trimethoprim/sulphamethoxazole and tetracycline. Though still at low levels, the major concern from our findings is the emerging resistance of enteric pathogens that was observed to quinolones (ciprofloxacin, nalidixic acid, norfloxacin) and gentamycin (Willie et al., 2011).

2.22 METHODS OF TESTING

The antibiotic sensitivity of bacteria can be assessed in a variety of ways according to individual preference, the constraints of cost, the nature of the bacterium, the member of strains requiring investigation and the degree of accuracy required. Most methods fall into one of three main categories.

Agar diffusion tests, in which the antibiotic is allowed to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organism.

Broth dilution tests, in which serial (usually two fold) dilutions of antibiotic, in a suitable fluid medium are inoculated with the test organism. The
highest dilution of the antibiotic to antibiotic to inhibit growth after overnight incubation is the minimum inhibitory concentration.

**Agar incorporation tests**, which are essentially similar to broth dilution tests except that the antibiotic dilutions are incorporated in an agar medium in a series of petridishes. These are spot inoculated with a number of test organisms, usually by means of a semi-automatic inoculating device.

Cefuroxime is a semisynthetic parenteral cephalosporin with a broad antibacterial spectrum. In this study, the antibacterial activity of the parenteral cephalosporin, cefuroxime, was compared with that of cephaloridine and cephalothin, which have been commercially available and widely used for over 10 yrs and with that of cefamandole, an analogue considerably more active against gram negative bacilli than cephaloridine and cephalothin (Eykyn et al., 1976).

The in vitro activity of cefuroxime was compared with that of cephaloridine, cephalothin and cefamandole against strains of gram negative bacteria isolated from clinical sources (Eykyn et al., 1976).

A combination therapy with an aminoglycoside plus a semisynthetic penicillin or cephalosporin commonly used in the treatment of life threatening infections caused by gram negative bacilli especially in immunocompromised patients (Anderson et al., 1978). These antibiotic combinations, shown to be synergistic both in vivo and in vitro (Jones and Packer, 1982) are used in place of single drug therapy with the aim of obtaining increased therapeutic efficacy as well as reducing the likelihood of emergence of resistance among infecting gram negative bacilli (Laethem, Lagast and Klastersky, 1983) B-lactam antibiotics are currently used widely in the treatment of infections caused by Gram-negative bacteria. These bacteria are surrounded by an outer membrane which contains
porin proteins that produce transmembrane diffusion channels (Yeshimura and Nikaido, 1985). Goodwin and Hill (1977) stated that many enterobacteria produce betalactamases, including cephalosporinases, cephalo sporin and cephalothin lyse such enterobacteria, but are destroyed after a few hrs and the organisers recommence logarithmic growth. Cefazolin appears to be more stable to cephalosporinases (Chan - tech and Grady, 1975).

Eng (1985) studied seven B-lactam antibiotics for both their antimicrobial activity and the degree to which they produced inoculum effect on *E.coli*, *K.pneumoniae* and *S.typhimurium*. Cefotaxime and Ceftriaxone were more rapidly bactericidal, caused only a moderate amount of filamentous forms and exhibited a modest inoculum effect, which Cefoxitin and Imipenem both were rapidly bactericidal and exhibited only a minimal to no inoculum effect. The inoculum effect did not correlate with drug stability during incubation with the bacteria. Inoculum effect on these species of the family Enterobacteriaceae appears to be a manifestation of increase in optical density secondary to the development of filamentous bacterial forms with an increase in bacterial mass during exposure to antibiotics which are rapidly bactericidal. These observations have a clear significance for the susceptibility testing of beta-lactam antibiotics when turbidity is used as a parameter to determine presence of bacterial growth.

Ampicillin at a 50 mg/ml concentration effects a doubling of the autolysis rate of cell envelops isolated from *K.pneumoniae*. This antibiotic increases two to three fold the de-polymerizing activity of both *K. pneumoniae* and *E.coli* crude extracts on a labelled peptidoglycan lipoprotein complex. Several other penicillins also activate autolysins (Fontana *et al*., 1977).
Lysis of bacterial cells treated with penicillin has been considered by many authors as a consequence of peptidoglycan cross linking inhibition (Blumberg and Strominga, 1974; Ghysen et al., 1975). E.coli can carry extrachromosomal resistance determinants (R factors) mediating constitutive synthesis of B-lactamase. The production of this enzyme renders the organisms less susceptible to the lethal action of penicillins and cephalosporins and may even lead to complete resistance to these antibiotics (Richmond et al., 1971).

Etiology and epidemiology of childhood diarrhoea in a given area is needed to plan preventive measures for diarrhoeal illness and develop practical guidelines. Morbidity and mortality caused by gastroenteritis decreased in relation to factors largely responsible for fall in infant mortality and morbidity from communicable diseases in developing countries. In 1993, 420.2 cases at 100,000 inhabitants were reported, most commonly affected are children of age 0-4 years. Incidence decreased to 338.5 cases at 100,000 inhabitants in 1997. Importance of oral rehydration solution in the treatment of diarrhoeal disease can be applied to all types of diarrhoea (Popa et al., 1998).

Conventional and molecular techniques (PCR) in 150 children less than 5 years of age admitted to Central Paediatric hospital, Gaza, Palestine were investigated. *Escherichia coli* O157:H7 7/150 (95%) , *Salmonella* spp 3/150(2%) , *Shigella* and *Salmonella* isolates were tested for their susceptibility to common antimicrobial agents and most of the isolates were resistant to ampicillin and trimethoprim (Abu-Elamreen et al., 2008).

The role of breastfeeding in preventing diarrhoea 98 Mexican children were followed by 2 years from birth. Diarrhoea in children less than 6 months who were not breastfed were 2-3 times greater than those in children of the same age who were fed human milk. Breastfed children remained free of diarrhoea for a
longer time than non breastfed children. Secretory Ig A milk antibody titres against glycine and extractable antigen of *C. jejuni* were high in Colostrum, decreased during the first month of breastfeeding and generally lasted throughout lactation (Ruiz-Palacios *et al.*, 1990).

A group of babies were observed during the first year of life for diarrhoea for fecal carriage of heat labile toxigenic bacteria. Half of the babies were breastfed for the first 6 months of life. There was no difference between groups (breastfed vs non breastfed) in babies with diarrhoea. Secretory antibody to toxin was found in 37% of colostrum and milk samples (Cushing *et al.*, 1982).

In a cohort study of 336 infants in Finland followed them from birth for the occurrence of acute diarrhoea. Breastfeeding over 6 months of age reduced the incidence of diarrhoea in the first year of life, as infants breastfed longer had diarrhoea in the second year of life. Prolonged breastfeeding reduced severity of diarrhoea in infants (Ruuska *et al.*, 1992).

In a longitudinal study of 13 breastfed and 14 formula fed infants over 6 months of life, salivary IgA, anti-*Escherichia coli*, anti beta lactoglobulin and anti poliovirus type 1 IgA and IgM in serum and saliva were evaluated. Salivary IgA was lower at one month in breastfed compared to formula fed infants but increased and was higher at six months than at 1 month (Avanzini *et al.*, 1992).
2.23 CHARACTERISATION OF BACTERIAL DIARRHOEAL PATHOGENS

PCR (Polymerase chain Reaction)

The technical advantages of the PCR based detection of EPEC strains offer a renewed opportunity to study the epidemiology of this organism in developing countries where EPEC is a major cause of infantile diarrhoea.

Enteropathogenic *Eschenichia coli* (EPEC) is a major cause of infantile diarrhoea among children in developing countries. Techniques to differentiate EPEC strains from non pathogenic strains and other *E.Coli* strains associated with diarrhoea have been confined to research laboratories. A probe to detect the EAF plasmid, called the EAF probe was developed to identify EPEC strains. Recently the EAF plasmid was found to encode inducible fimbrial structures called bundle forming pili (BFP). The BFP gene (bfp-4) sequences from different EPEC strains show a high degree of homology and appear to be specific.

In a study a probe derived from the structural gene of the BFP was used to determine the distribution of BFP among EPEC strains. The BFP gene probe proved to be more specific than the EAF gene probe in the identification of EPEC strains. No cross-reactivity with other entenic *E.coli* was noted with the BFO gene probe under high stringency conditions. A PCR procedure has previous by been applied to identify gene sequences from pathogenic enteric *E.coli* strains. The procedure relies on detecting DNA sequences of interest amplified by a set of synthetic oligonucleotide primers.

All bacterial strains examined by PCR were grown on tryptic soy agar overnight at 37°C. DNA was extracted from bacteria by resuspending the bacteria in a small volume (1 ml) of sterile deionised water and boiling the suspension for
10 min. Primers capable of amplifying an internal 326 bp region of the gene sequence encoding the mature 19-KDa BFP protein was constructed. The primers used were based on the published bfp A gene sequence. DNA was amplified by 29 cycles (each cycle consisted of 30s of denaturation at 94°C, 1 min of annealing at 56°C and 2 min of primer extension at 72°C). The amplified DNA product was resolved by agarose gel electrophoresis and visualised under UV transillumination after the gel was stained with ethidium bromide.

In a study in Taiwan PCR tests detected 6 EPEC (4%) isolates from children below 5 years of age. 150 strains of E.Coli isolates from children with sporadic diarrhoea treated at National Taiwan Hospital were studied for 1 year. EPEC, an important paediatric diarrhoeal pathogen, employs multiple adhesins to colonize the small bowel and produces characteristic "attaching and effacing" (A/E) lesions in small intestinal enterocytes. EPEC adhesins that have been associated with A/E adhesion and intestinal colorization include BFP, ESP A filaments and intimin. BFP are involved in bacteria - bacteria interaction and microcolony formations. 2 sets of PCR primers were used to increase sensitivity and specificity to detect EPEC one detects bfp gene and other detects EAF plasmid. BFP is encoded on EAF plasmids, which share extensive homology among various EPEC strains. The importance of the EAF plasmid in human disease was shown by Levine et al (1993). The EDF plasmid has been reported to control the expression of the eaCA gene product, intimin. The eacA gene has also been used as a target for the detection of EPEC. In a similar study undertaken in Malaysia by Irene Lah, 32 strains of EPEC isolated from Hospital Mini, Siriwak were examined and further characterised by various molecular techniques. These techniques include plasmid profiling, antimicrobial resistance, resistance and virulence genes detection by multiplex PCR, RAPD, ERIC and PFGE genomic fingerprinting. All the strains studied were found to exhibit antibiotic resistance to
12 antibiotics. 13 EPEC isolates were shown to encode Ampicillin resistance by means of the blaTEM gene, none of the EPEC isolates showed the presence of the Sip B/C, CMIA / tetr and blasp-1 genes. The plasmid profiles obtained ranged in size from 1.8 MDa to 57MDa. Two EPEC isolates analysed by PCR were confirmed to be the D157:H7 serogroup as determined by agglutination tests with specific anti-era (Lee-Jene Teng et al., 2004).

Multiplex PCR and O & H typing techniques were used for detection of enteropathogenic Escherichia coli (EPEC). EPEC are defined as intimin containing diarrhoegenic E.coli isolates. The detection of this group of diarrhoegenic E.coli is based on serological and newly developed molecular methods. 500 E.coli isolates were used in this study and subjected to serological typing and a multiplex PCR. Multiplex PCR revealed 41 isolates are EPEC of which 27 were typical and 14 isolates were a typical (Amir Dasti et al., 2009).

A multiplex PCR assay was evaluated for diagnosis of diarrheagenic E.coli in patients with diarrhoea. 1.7% of the isolates were EPEC by proteinase K buffer method (Khairunnissa et al., 2007).

Real time PCR detection of Escherichia coli, Nucleic acid testing (NAT) is the method of choice for detection and quantification of a wide range of micro-organisms. Real time PCR assays are increasingly being used for detecting and quantitating bacterial populations that are present in relatively low numbers, which is not possible with northern hybridization, using the slot blot technique. This application has been recently included in studies of the large intestine. This is done to investigate the effects of different types of feeding on bacterial populations.
DNA extraction from bacterial cultures

DNA is isolated from pure cultures of bacteria using a modified method based on the Qiagen DNA Blood mini kit. Bacterial cells resuspended in 450 ml of deionised water to which 50 ml of lysozyme (50 mg ml\(^{-1}\)) was added and incubated at 37°C for 30 min. Twenty five microlitres of proteinase K (20 mg ml\(^{-1}\)), 50 ml of 20% sodium dodecyl sulphate and 0.1 mm glass beads (350 mg) were added prior to mechanical disruption (2 min) using a mini bead beater. The bead heating step was repeated after 10 min incubation at 60°C and the cell debris was removed by centrifugation (5000g, 3 min). DNA in the supernatants was then purified using the Qiagen DNA Blood mini kit according to manufacture instructions.

DNA extraction from faecal material

Fresh faeces was homogenised (10 w/v) in 0.1 M sodium phosphate buffer (pH 6.5). DNA was extracted from 2ml of the faecal slurry using the Qiagen DNA stool kit, the cells were collected by centrifugation at 20,000 g (10 min) and resuspended in 1.4 ml of ASL lysis buffer. Glass beads (350 mg) were added and beaten for 2 min at maximum speed using a mini bead beater. The suspension was incubated at 95°C for 5 min. After centrifugation (5000g, 2 min) to remove cell debris, the supernatant was transferred to a clean vial and an inhibitax tablet added to remove DNA damaging substances and PCR inhibitors.

PCR Primers

1. Determining the specificity of each primer set and the optimum annealing temperature was done using a blaster cycler gradient PCR machine and Techne Genius PCR machine.
Real time PCR

Quantitation of bacterial DNA is done using an I cycler Real time PCR Detection system. Each reaction is done in duplicate in a volume of 20 ml in 96-well optical grade PCR plates, sealed with optical sealing tape. Amplification reactions are done with supermix containing 3 mM MgCl$_2$, 20mM Tris Hcl (pH 8.4), 50 mM Kcl, each dextranucleotide triphosphate at a concentration of 200 mM. DNA polymerase mixed with the selected primer set at a concentration of 0.5 mM for each primer and 16 ml of the respective DNA. Amplifications were done with the following temperature profiles: one cycle at 95°C for 3 min, 38 cycles of denaturation at 95°C (30s) followed by 30 s at the appropriate annealing temperature, and one final cycle at 95°C (30s).

A widely used method for characterisation is PCR. In PCR, a pair of primers (20-40 bases) is used for selective amplification and detection of a certain DNA sequence in a target organism. PCR primers have successfully been developed for all categories of diarrhoegenic *E.coli*. PCR can be used for both diagnosing and typing *E.coli* strains. Advantages of PCR include high sensitivity, specificity and appropriate rapidity in the detection of target DNA templates. In diagnostics, PCR is commonly used for detecting different virulence associated genes of *E.coli*, such as toxin and adherence associated genes. PCR is also widely used in sub typing. The RAPID, a PCR method is sensitive and efficient. A pair of short primers is bind radomly to the DNA sequence and amplified into bands. Other techniques such as enterobacterial repetitive intergenic consensus (ERIC) PCR (Gison *et al.*, 1984; Hulton *et al.*, 2001).

Diarrhoegenic *E.coli* were among the first pathogens for which molecular diagnostic methods were developed. Molecular methods, especially polymerase chain reaction (PCR) are nowadays considered the most reliable technique to
characterise diarrhoegenic *E.coli* str. The importance of enteroaggregative *Escherichia coli* (EAggEC) as a possible aetiological agent of acute diarrhoea among children in Calcutta, India, was investigated. Simultaneously the use of a previously described PCR diagnostic system was assessed for its ability to identify EAggEC infection. *E. coli* strains isolated during a 1-year case-control study from faecal samples of 388 children aged <5 years, with or without diarrhoea, were examined for EAggEC by HeLa cell adherence assay in parallel with a PCR assay with primers generated from an EAggEC DNA probe. A blind comparison was made between the two methods to determine their diagnostic potential. *E. coli* isolates that adhered to HeLa cells in an aggregative pattern were the sole isolates significantly more often in 254 cases (9%) than in 134 control (2%) children. Age stratification showed that EAggEC were isolated more frequently from children aged <36 months. The EAggEC isolates belonged to several O serogroups and showed multiple drug resistance. Both methods were positive for 26 samples, nine samples were positive by PCR alone and seven samples were positive by culture alone, thus indicating a 78% sensitivity and 97% specificity for the PCR assay. EAggEC is an important aetiological agent of acute diarrhoea among infants in and around Calcutta, and the PCR diagnostic system may be useful to identify such infection in epidemiological studies (John et al., 2005).

Real time PCR has revolutionized the way clinical microbiology laboratories diagnose human pathogens. This testing method combines PCR chemistry with fluorescent probe detection of amplified product in the same reaction vessel. In general, both PCR and amplified product detection are completed in an hour or less, which is considerably fast than conventional PCR and detection methods. hence, for some time this technology was referred to as rapid cycle real time PCR. Other descriptions of real time PCR in the early literature included homegenous PCR and kinetic PCR.
Sensitive and specific detection is possible with real time PCR by using novel fluorescent probe technology probes.

A widely used method for characterisation is PCR. In PCR, a pair of primers (20-40 bases) is used for selective amplification and detection of a certain DNA sequence in a target organism. PCR primers have successfully been developed for all categories of diarrhoegenic E.coli. PCR can be used for both diagnosing and typing E.coli strains. Advantages of PCR include high sensitivity, specificity and appropriate rapidity in the detection of target DNA templates. In diagnostics, PCR is commonly used for detecting different virulence associated genes of E.coli, such as toxin and adherence associated genes. PCR is also widely used in sub typing. The RAPID, a PCR method is sensitive and efficient. A pair of short primers is bind radomly to the DNA sequence and amplified into bands. Other techniques such as enterobacterial repetitive intergenic consensus (ERIC) PCR (Gison et al., 1984; Hulton et al., 2001).

Diarrhoegenic E.coli were among the first pathogens for which molecular diagnostic methods were developed. Molecular methods, especially polymerase chain reaction (PCR) are nowadays considered the most reliable technique to characterise diarrhoegenic E.coli strains from one E.coli pathogen to another (Nataro et al., 1998).

**Behaviours that increase the risk of diarrhoea**

A number of specific behaviours help enteric pathogens to spread and thus increase the risk of diarrhoea. These include:

- Failing to breast feed exclusively for the first 4-6 months of life. The risk of developing severe diarrhoea is many times greater in non breast fed infants
than in infants who are exclusively breast fed, the risk of death from diarrhoea is also substantially greater.

- Failing to continue breast feeding until at least one year of age.

- Using infant feeding bottles. These easily become contaminated with faecal bacteria and are difficult to clean. When milk is added to an unclean bottle, it becomes contaminated.

- Failing to sterilise infant feeding bottles.

- Contamination of water used for preparing formula milk.

WHO aimed at describing the growth of healthy breastfed infants living in good hygeine conditions was conducted between 1997 and 2003 in 6 countries: Brazil, Ghana, India, Norway, Oman and United States. A longitudinal follow up of 882 infants was done from birth to 24 months. The study populations lived in socio-economic conditions favourable to growth. The WHO child growth standards were derived from children who were raised in environments that minimised constraints to growth such as poor diets and infection. In addition, mothers followed healthy practices such as breastfeeding and not smoking after pregnancy. The standards explicitly identify breastfeeding as the biological norm and establish the breastfed child as the normative model for growth and development. They significantly strengthen health policies and public support for breastfeeding. The pooled sample from 6 participating countries allowed the development that child populations grow similarly across the world's major regions when their health and care needs are met. WHO standards provide a better tool to monitor the rapid and changing rate of growth in early infancy. Healthy environment and follow recommended feeding practices have strikingly similar growth patterns.(De Qnis et al., 2009).
3. MATERIALS AND METHODS

The present study is based on the feeding practice, bacteriological diagnosis of hospitalized (in patients) and non-hospitalized (out patients) infants aged 0-6 months suffering from diarrhoea during the period of 3 years from June 2007 to April 2010. Infants with symptoms like secretary diarrhoea, vomiting, dehydration, cholera and gastroenteritis attending the hospitals formed the study group. Faecal specimens and rectal swabs were collected and transported immediately to the laboratory in peptone water and alkaline peptone water in sterile containers. Control group were normal school and resident children.

3.1 STUDY POPULATION

Study population included infants in the age range of 0 - 6 months. The patients were either hospitalized or non-hospitalized children. Hospitalized patients were the in-patients in the hospitals and the non-hospitalized patients were those attending outpatient department of the hospitals.

The hospitals included in this study were Egmore Government Children's hospital in Chennai (Urban area) and 2 private clinics in Naickenkuppam village (rural area).

Patient record

The hospital records of patients infected with diarrhoea were reviewed. Information recorded from each chart included age, sex, socio-economic status, clinical diagnosis and microbiological outcome.
3.2 FIRST PHASE OF THE STUDY

STUDY DESIGN

The study groups were

1. Breast fed infants suffering from diarrhoea in the age range of 0-6 months in urban and rural areas.
2. Bottle fed infants suffering from diarrhoea in the age range of 0-6 months in urban and rural areas.
5. Breast fed and bottle fed infants - healthy without diarrhoea.

3.3 SAMPLES

300 Children under the feeding age of 0-6 months were selected for the study. 200 samples were selected from the hospitalized and non-hospitalized children suffering from diarrhoea and the remaining 100 control samples from residential areas. Both male and female children belonging to the age group of 0-6 months were selected.

Table 1

<table>
<thead>
<tr>
<th>Name of centre</th>
<th>Breast-fed</th>
<th>Bottle-fed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Govt. Children Hospital, Egmore, Chennai City</td>
<td>35</td>
<td>82</td>
<td>117</td>
</tr>
<tr>
<td>Private Clinics, Naickenkuppam Village</td>
<td>37</td>
<td>46</td>
<td>83</td>
</tr>
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</table>
Table 2

DISTRIBUTION OF CHILDREN IN CONTROL GROUP (n=100)

<table>
<thead>
<tr>
<th>Area of Study</th>
<th>Name of Centre</th>
<th>Breast-fed</th>
<th>Bottle-fed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chennai City Residents</td>
<td>Residents</td>
<td>22</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>Naickenkuppam Village</td>
<td>Residents</td>
<td>33</td>
<td>7</td>
<td>40</td>
</tr>
</tbody>
</table>

Collection of Stool Sample

Samples such as rectal swabs and stool specimens were collected from the infants.

Macroscopic appearance of Stool sample

The appearance, consistency, colour and odour of the stool samples were observed. Presence of blood and mucus denoting dysentry cases were also noted.

Processing and Isolation of bacteria

The media used for isolation were Nutrient agar (NA), Eosin Methylene Blue agar (EMB), MacConkey agar (MAC), Cetrimide agar (CA), Salmonella Shigella agar (SS) and Thiosulphate citrate Bile Salt sucrose Agar (TCBS).

One loopful of stool samples were streaked on various selective media and incubated at 37°C overnight.
3.4 IDENTIFICATION OF BACTERIAL DIARRHOEAL PATHOGENS

After 24 hrs, only the isolated colonies were taken, inoculated in peptone water and alkaline peptone water and incubated at 37°C for 4 hrs. Preliminary tests like Gram staining, hanging drop, catalase and oxidase tests were performed as per the standard procedure.

3.5 CULTURAL AND BIO-CHEMICAL CHARACTERISATION

CULTURAL

Peptone Water

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<tr>
<td>Sodium chloride</td>
<td>5gms</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td><strong>pH</strong></td>
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</tr>
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</table>

Autoclave at 121°C for 15 minutes.

Alkaline Peptone Water

<table>
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</thead>
<tbody>
<tr>
<td>Peptone</td>
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</tr>
<tr>
<td>Sodium chloride</td>
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<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td><strong>pH</strong></td>
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Autoclave at 121°C for 15 minutes.
Cary and Blair Medium

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</thead>
<tbody>
<tr>
<td>Sodium thioglycolate</td>
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</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>1.1gm</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>gm</td>
</tr>
<tr>
<td>Agar</td>
<td>5gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>991ml</td>
</tr>
<tr>
<td>pH</td>
<td>8.4</td>
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Nutrient Broth

<table>
<thead>
<tr>
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<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
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</tr>
<tr>
<td>Meat extract</td>
<td>10gm</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 gms</td>
</tr>
<tr>
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<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
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</table>

Autoclave at 121°C for 15 minutes.

Nutrient agar

<table>
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<tbody>
<tr>
<td>Peptone</td>
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</tr>
<tr>
<td>Beef extract</td>
<td>10gm</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 gm</td>
</tr>
<tr>
<td>Agar</td>
<td>15gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Autoclave at 121°C for 15 minutes.
MacConkey Agar

Peptone : 20gm
Sodium taurocholate : 5 gm
Agar : 20gm
Distilled water : 1000ml
Neutral red(2% in 50%ethanol) : 3.5ml
Lactose,10% aqueous solution : 100ml
pH : 7.5

autoclave at 115°C for 15 minutes.

Eosin Methylene Blue Agar

Peptone : 10gm
Lactose : 10gm
Dipotassium Hydrogen phosphate : 2 gm
Agar : 15gm
Eosin –Y : 0.4gm
Methylene blue : 0.06gm
Distilled water : 1000ml
pH : 7.1

Deoxycholate Citrate Agar

Meat extract : 5 gm
Peptone : 5gm
Agar : 22.5gm
Neutral red (25 in 50%ethanol) : 1.25 ml
Lactose : 10gm
Distilled water : 1000ml
pH : 7.4

Steam sterilization at 100°C

**Salmonella Shigella Agar(SS)**

Salmonella Shigella agar is a selective medium which is used for the isolation of *Salmonella* species and many strains of *Shigella* species.

- Beef extract : 5gm
- Peptone : 5 gm
- Lactose : 10gm
- Bile salts : 8.5gm
- Sodium citrate : 8.5gm
- Ferric citrate : 1gm
- Brilliant green : 0.33gm
- Neutral red : 25gm
- Agar : 13.5gm
- Distilled water : 1000ml
- pH : 7.4

**Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS)**

This is the selective medium for cultivation of Vibrios and *Vibrio cholera*

- Yeast extract : 5gm
- Peptone : 10gm
- Sodium chloride : 10gm
- Sodium thiosulphate : 10gm
Sucrose : 20gm
Bromothymol blue : 0.04gm
Ferric citrate : 1gm
Thymol blue : 0.04gm
Sodium citrate : 10gm
Agar : 15gm
Oxbile : 8gm
Distilled water : 1000ml
pH : 8.6

Heat at 100°C do not autoclave

**SORBITOL MAC CONKEY AGAR**

**Composition:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin peptone</td>
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</tr>
<tr>
<td>D-sorbitol</td>
<td>10gms</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5gms</td>
</tr>
<tr>
<td>Bile salts</td>
<td>1.5gms</td>
</tr>
<tr>
<td>Meat peptone</td>
<td>1.5gms</td>
</tr>
<tr>
<td>Caesin peptone</td>
<td>1.5gms</td>
</tr>
<tr>
<td>Neutral red</td>
<td>30mg</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>1 mg</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 mg</td>
</tr>
<tr>
<td>Demineralised H2O</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

50gm of the medium was suspended in 1000ml of demineralised water.

Completely dissolved by heating to boiling.

Sterilised by autoclaving at 121°C for 15 min
**Mueller Hinton Agar**

- Beef infusion : 300ml
- Caesin hydrolysate : 17.5gm
- Starch : 1.5gm
- Agar : 10gm
- Distilled water : 1000ml
- pH : 7.4

Autoclave at 121°C for 20 minutes.

### 3.6 SUGAR FERMENTATION TESTS

**Principle:**

Energy is generated by bacterial cells from nutrients through fermentation. Fermentation partially oxidizes the substrate and generates a small amount of ATP. Sugar fermentation patterns are used in the identification of enteric bacteria. Different bacteria ferment a wide variety of sugars. Fermentation pattern can be determined for a series of different carbon sources (sugars) and this serves as a central part in the identification process. Acid products, produced from sugar fermentation causes a noticeable colour change in the pH indicator in the medium. Alkaline reactions can be seen by non-fermentative hydrolysis of amino acids in peptone present in fermentation media. Gas production, H2 in particular can be detected by placing a small inverted durham tube in the test medium. Trapped gas can be seen as a bubble in the durham tube.
**Composition**

Purple broth base:

- Peptone : 1%
- Beef extract : 0.1%
- Na Cl : 0.5%
- Bromocresol Purple : 20ug/ml
- Sugar : 1%
  - (glucose, sucrose, lactose Mannitol, Arabinose, Dulcitol, Raffinose, Rhamnose)

### 3.7 BIOCHEMICAL TESTS

**Methyl Red/Voges Proskauer Test:**

**Composition:**

Glucose phosphate Broth:

- Buffered peptone : 7 gm
- Dextrose : 5 gm
- Dipotassium phosphate : 5 gm
- Distilled water : 1000ml
- Final pH : 6.9+0.2