2. REVIEW OF LITERATURE

Diarrhoea or gastroenteritis is an old clinical problem for mankind as reference to the disease are found in ancient Palestinian and Biblical writings. Since the time of Hippocrates, the term diarrhoea has been used to designate the increased frequency, fluidity and volume of faeces (Levine and Edelman, 1979). The term gastroenteritis denotes abdominal cramps, blood and/or mucus in the faeces with or without vomiting. Diarrhoea (taken from the Greek meaning "to flow through") is an important cause of morbidity and mortality in developing and under developed countries.

Diarrhoeal disease constitute a significant social, economic and medical challenge to mankind. With improvement in socioeconomic status and environmental sanitations, its incidence has considerably declined in developed countries during the last few decades. However, in lesser developed parts of the world, the diarrhoeal problem continues unabated.

There is a direct correlation between diarrhoea and infant death rates in a community (Mata, 1981). In India, over 500 million episodes of diarrhoea occur each year and about 5 million of these may require treatment at health facilities (Banerjee, 1985).

2.1 Route of transmission

An insanitary environment is a major factor in the spread of diarrhoeal organisms e.g. bacteria, virus or parasite. The route of infection is either through direct transfer from animal or human faeces or through water and food (Hobbs and Gilbert, 1978).
Man may act as the reservoir for certain etiological agents such as enterotoxigenic \textit{E. coli} (ETEC), \textit{Shigella}, \textit{Vibrio cholerae}, \textit{S. typhi}, \textit{Giardia lamblia} and \textit{Entamoeba histolytica} (Feachem, 1984), while for others namely \textit{Campylobacter jejuni}, \textit{Salmonella} and \textit{Yersinia enterocolitica}, animals are the main reservoir (Butzler and Skirrow, 1979; Swaminathan et al., 1982; Hobbs and Roberts, 1987).

2.1.1 Water contamination

Water is a primary source of diarrhoeal epidemics as it is used by a large populations from a single source. Many pathogenic organisms may enter water from faeces of sick or healthy excretors, both human and animal. Sewage has led to epidemics and/or pandemics of water-borne diseases such as bacillary dysentery, Salmonellosis, cholera, typhoid and paratyphoid fevers.

Water-borne outbreaks of gastrointestinal illness are a threat to public health throughout the world. An outbreak of gastroenteritis caused by \textit{E. coli} in adults occurred at a conference centre near Washington, D.C. in April 1967, the cause being a contaminated non-chlorinated water system. Enteropathogenic \textit{E. coli} serotype 0111:B4 was identified both in the water and faecal specimens of 14 participants (Schroeder et al., 1968).

The role of contaminated water in relation to diarrhoea has been a topic of discussion by WHO in 1976 and 1981. Rosenberg et al. (1977) reported a water-borne epidemic of diarrhoea in which more than 200 staff members and 2000 visitors to an American national park were affected. Enterotoxigenic \textit{E. coli} serotype 06:K15:H16 was isolated from 20 out of 49 sick persons and the water sample from the park.
During monsoon, incidence of cholera occurs regularly after every heavy rain. The climate affects pollution of water. Higher counts are observed after rains (Hobbs, 1982).

Potable water has been implicated in several large outbreaks of Campylobacter enteritis. In Sweden about 2000 persons (20%) of the community at risk were described by Mentzing (1981). An outbreak involving about 3000 (19%) residents of Bennington, Vermont was reported by Vogt et al. (1982). In England and Iceland, C. jejuni has been isolated from both salt and fresh water sources (Blaser et al., 1983).

An assessment of 24 studies from around the world by Hughes (1983) suggested that an improvement in bacteriological quality of water alone, reduces diarrhoeal disease on an average by 30 per cent.

Two water-borne outbreaks of rottavirus diarrhoea involving about 12,000 adults in China during 1982 and 1983 were reported by Tao et al. (1984).

A study carried out in Madras, has shown that water quality is an important determinant for diarrhoeal disease in children, under 3 years of age while it acts as a strong determinant in children above 3 years (Singhi and Kumar, 1985).

Puttalingamma et al. (1985) studied the incidence and distribution of different types of bacteria found in Mysore municipal water supply. The numbers and types of bacteria from various sources fluctuated. However, higher counts were obtained after rain. The more prevalent bacteria were Enterobacter and Pseudomonas.
Zmirou et al. (1987) analysed weekly untreated ground water samples in a follow up study of 52 French alpine villages and suggested that any level of indicator bacteria above zero was associated with excess of acute gastro-intestinal disease.

The chlorination of drinking water in Britain was initiated by Alexander Houston in 1905 during a typhoid epidemic in Lincon, and this development has abolished water-borne disease in U.K. and other countries (Hobbs and Roberts, 1987).

2.1.2. Food contamination

Hall et al. (1967) examined 490 food samples for total aerobic counts and types of coliform organism in food articles namely frozen desserts, cheese and cheese products, baby foods, fish and sea foods, dry cereals and mixes, raw and frozen vegetables, prepared and convenience foods, raw meats and sandwiches. EPEC serotype 026:B6 was isolated from cubed beef steak and pork liver while serotype 055:B5 was isolated from frozen gravy and sliced beef. Only 0.6% of the food specimens contained EPEC, whereas 6% of 219 specimens of faeces from healthy food handlers contained one or more strains of EPEC.

Hunter et al. (1967) proved that the consumption of food contaminated with *P. aeruginosa* gave rise to disturbances in the GI tract, with colicky pain, diarrhoea and usually vomiting. Baljer and Barrett (1979) demonstrated that all the seven strains of *P. aeruginosa* isolated from human food stuff were producing enterotoxins. A mass food poisoning caused by consumption of stew contaminated with it has been reported by Rokoszewska et al. (1980).
P.aj and Lamba (1972) isolated *S. aureus* from the faecal samples of 21 persons involved in food poisoning and from the samples of the suspected food (curd) and the container used for storing it.

The first well documented outbreak of food-borne illness caused by *E. coli* in the United States was reported by Marrier et al. (1973), at least 387 persons were involved in 107 episodes of gastroenteritis. Camembert cheese imported from France with an attack rate of 94% was involved. *E. coli* 0124 was isolated from 14 of 49 faecal samples and from 25 of 75 cheese samples. Only 2 of 75 yielded *E. coli* 0125. Several of these samples contained *Enterobacter* and *Hafnia* strains which cross reacted with *E. coli* 0112:B11 antisera. Counts revealed $10^5$ to $10^7$ *E. coli* 0124 per gram of cheese.

In contrast, in a study by Singh and Ranganathan (1974) in India, 30 out of 128 raw milk samples contained pathogenic *E. coli* serotypes. These were also found in ice cream, butter and cheese.

Between 1969 and 1972, *E. coli* accounted for less than 2% of outbreaks of food borne illness in the United States and less than 6% of total cases of bacterial gastroenteritis (Mehlman et al., 1976). Gilbert and Parry (1977) reported that between 8000-12,000 cases of food poisoning were notified each year in England and Wales.

Sack et al. (1977) obtained 240 isolates of *E. coli* from foods of animal origin and about 8% of the strains produced LT. These strains came from soft cheese, hamburger, crab meat and sausage.

Joshi (1977) reported a high incidence of *E. coli* associated food poisoning outbreak in India. Food poisoning outbreaks due to
Aeromonas hydrophila in India and Ethiopia were described by Hobbs and Gilbert (1978). They further stated that in food-borne diarrhoeal outbreaks around the world, the food items implicated were poultry, meat and meat products, milk and milk products, fish, rice and other cereal formulations, egg and egg products, fruit and vegetables.

Todd (1978) compared the data for food-borne illness from six countries. The number of outbreaks ranged from 48 in Australia to 6,109 in Japan over a five years period. Mostly Salmonella, Staphylococcus aureus and Clostridium perfringens were the agents primarily responsible for gastroenteritis.

In 1978, beef obtained from a food service establishment and subjected to improper holding and cooking temperatures, and contaminated equipment resulted in a food-borne outbreak of E. coli involving 35 persons (Anonymous, 1979).

Pseudomonas and Acinetobacter were found to be mainly responsible for typical spoilage of fresh food during refrigeration (Livingston and Chang, 1979). In general these micro-organisms must be present in large numbers, usually over 10⁷ cells/g before it develops a typical spoilage aroma or visual degeneration.

Reis et al. (1980) surveyed 120 packages of hamburger, sausage and "Keebe" obtained from a local supermarket in Brazil. Of 1,200 E. coli isolates, ETEC were found in 5% of Keebe, 7.5% of hamburger and 10% of sausage samples.

Aggarwal et al. (1982) studied bacterial contamination in 150 samples of weaning foods fed to children, of which 50% yielded
I t. ive cultures of E. coli indicating faecal contamination; 15.3% K. pneumoniae, 18.7% P. aeruginosa, 14% S. faecalis, 2% Proteus and 0.66% Citrobacter.

Tendency for an increase in outbreaks of Salmonella, S. aureus and B. cereus food poisoning during summer has been noticed. B. cereus food poisoning was associated with cooked rice, usually fried in Chinese restaurants (Hobbs, 1982). The spores of B. cereus survive normal cooking and vegetative cells multiply during warm storage, particularly in large bulks of rice.

An extensive international outbreak of S. eastbourne food poisoning in North America in 1974 involved chocolate candy containing below one Salmonella cell per gram (Craven et al., 1975). Similarly in the 1975 outbreak, minced beef contained very few viable cells per 100 g of S. newport. However, the number of organisms required for clinical infection depended on the virulence of the organism, age and general health of the person and possibly many other factors.

Foods of animal origin, particularly meat, poultry and unpasteurized egg products are considered to be the primary source of human salmonellosis (WHO, 1976). Cross contamination of cooked foods from raw ingredients, kitchen utensils and surface is frequent responsible for salmonellosis. Occasional outbreaks have been traced to contaminated milk powder, dried egg, carmine red dye, dried yeast, vegetable protein extract, chocolate candy and even apple cider (WHO, 1976).
In industrialized countries, intensive farming of poultry and other animals for food has exacerbated the problem of salmonellosis. Egg shells become contaminated when the eggs are laid and also when they come in contact with nest litter materials or faecal droppings. As eggs cool, organisms on their surface can be drawn through the cell or they can penetrate the shell and membrane later (Bryan, 1982).

Simultaneous isolation of _S. stanley_ and _S. oranienburg_ from an outbreak of food poisoning at Maldives island has been reported by Ray _et al._ (1983).

In the four Atlantic provinces of Canada and Ontario during 1984, more than 2,000 cases of _S. typhimurium_ food poisoning occurred due to consumption of cheddar cheese (Bezanson _et al._, 1985).

Wafa _et al._ (1986) reported 8% occurrence of _Salmonella_ in Iraqi milk products. Positive samples included ice cream (10.9%), Kishfa (10%), Gaymu (7.5%), Cheese (6.6%) and Yogurt (1.6%). Out of 15 serotypes isolated, _S. typhimurium_ and _S. infantis_ occurred with the highest frequency.

Studies of travellers diarrhoea (Merson _et al._, 1976; Tjoa _et al._, 1977) have shown that contaminated food and water are the most likely vehicles for _Campylobacter_ infection. Chicken eaten raw by military recruits during a training exercise was implicated in an outbreak in the Netherlands (Brouwer _et al._, 1979).

In England and Wales, unpasteurized milk was identified as the vehicle of _C. jejuni_ infection in 13 outbreaks involving about 4500 persons during 1978 to 1980 (Porter and Reid, 1980; Robinson and Jones, 1981). To demonstrate the disease potential of
Campylobacter, volunteers took experimentally contaminated milk with 500 colony forming units of \textit{C. jejuni} and consequently developed typical illness (Robinson, 1981).

Poultry, eggs, meat and their products are frequent vehicles for food-borne \textit{Campylobacter} enteritis (Bryan, 1982). The role of food handlers in transmitting infection is not known. There was no transmission of \textit{Campylobacter} to food prepared by two cooks who were asymptomatic excretors (Norkrans and Svedham, 1982). However, \textit{C. jejuni} was isolated from the hands of another food handler who was ill with \textit{Campylobacter} infection. Salad, prepared by him was probably the vehicle of infection (Finch and Blake, 1985). In the United States, \textit{Campylobacter} associated outbreaks have been reported only among persons who prefer to drink raw milk (Finch and Blake, 1981).

A variety of bacterial pathogens have been reported to have caused gastroenteritis on airlines. In 23 reported outbreaks, \textit{Salmonella} was the most common agent followed by \textit{Staphylococcus} and \textit{Vibrio} species (Tauxe et al., 1987). There were different types of food vehicles, sandwiches, chicken, oysters, egg salad, custard, ham and cold salads.

\textit{Y. enterocolitica} and related organisms have been isolated from a variety of foods, especially of animal origin (Lee, 1977). Chocolate milk was the vehicle in first major outbreak (Black et al., 1978). In another outbreak, reconstituted powdered milk and turkey chow-mein were the vehicle of transmission (Shayagani et al., 1983). \textit{Y. enterocolitica} is capable of growing in food stored at 4°C (Bercovier and Moller, 1984).
Clostridium perfringens is a common contaminant of raw food ingredients and the spores of most strains may survive and/or be activated by the heat during cooking. These activated spores germinate and multiply during storage of cooked food which is left at room temperatures for several hours before consumption (Hobbs, 1979).

2.1.3 Food hygiene

Foster (1968) emphasised that the sanitary measures in a food service institution were of much greater significance than those in family homes because of the large number of customers at risk.

Hobbs and Gilbert (1978) defined food hygiene as the sanitary science which aims at producing food of good keeping quality and safe for the consumer.

Based on various studies, Bryan (1979) concluded that factors leading to food poisoning were inadequate cooking, warm storage, improper reheating and lack of cleaning of kitchen or of processing equipment, ingestion of contaminated raw food or other ingredients and cross contamination of food in food service establishments.

Kumar (1979) stated that hygiene is especially important in food service institutions since non-observance could result in large scale damage to health. Jolly (1980) reported that though the number of people in India who depend on hotels and restaurants for daily meals is smaller as compared to Western countries, yet fairly large number of people visit these frequently, hence the sanitary conditions at such institutions are of paramount importance.
2.1.3.1 Personnel

Harwood and Minch (1951) cautioned against the obvious contamination of food with organisms like coliforms, haemolytic *Streptococci*, *Staphylococci* and *Shigella*, which are commonly present on the hands of food handlers. Leger et al. (1975) reported that through hand washing with an effective disinfectant would eliminate haepatitis A virus particles from persons with asymptomatic cases. Hobbs and Gilbert (1978) emphasised the cleanliness of food handlers and suggested that their hands should be washed with plenty of soap and water, and nails be kept scrupulously clean. Further they observed that washing with soap and water were effective in reducing or removing coliform organisms acquired from food or water. Cuts and burns and other raw surfaces harbour *Staphylococcus*.

The usual source of *Shigella* in food-borne outbreaks is a human excretor. This person touches a food that is not subsequently cooked but held for several hours within a temperature range favourable for *Shigella* multiplication (Bryan, 1982).

2.1.3.2 Dish washing

Higgins and Hobbs (1950) surveyed 30 catering establishments and studied the washing technique used for utensils. Satisfactory bacteriological results were obtained from a group of hotels where hot water was used for cleaning. Sixty three per cent of the articles attained public health standard of 100 or less organisms per utensil. There was also a corresponding absence of respiratory and intestinal organisms.

Kahlon (1973) surveyed the fresh washed food utensils from canteen and cafe of an institution in Punjab (India) for bacterial
contamination and noticed a very high microbial count on the washed dishes. Adequate cleaning and bacteriocidal treatment was recommended.

Royce and Marianna (1975) attributed the high coliform count on utensils to dirty tank water, wipe-cloth and faulty handling of dishes by the employees. E. coli was found in 80% samples analysed, which indicated contamination with faecal matter. A significant difference in the presence of coliform and total count was evident after the introduction of improved technique of washing.

2.1.4 Indian sweets and salty snacks

Many kinds of traditional sweetmeat products are made and sold under varying conditions in India. Vatlewar et al. (1970) observed that nearly 47% of the sweetmeat samples in the market of Bombay (India) contained enteropathogenic bacteria and only 35% were free from such organisms. Mokashi et al. (1970) reported that only 5% of the ice cream samples sold in Bombay were free from bacteria and remaining showed coliforms of faecal origin. Most of the E. coli strains isolated were found to be diarrhoeagenic type. According to Kamat and Sulebele (1974), Pedha available in Bombay city was contaminated with Staphylococcus aureus.

Ghodeker et al. (1974) analysed 245 samples of Indian milk products, collected from different parts of the country. Higher bacterial and fungal counts were noted in Khoa, as compared to Burfi and Pedha. A variety of microorganisms such as Micrococcii, Sarcina, Bacillus, Coliforms, Staphylococci, Streptococci and Lactobacilli were isolated from the samples.
Singh et al (1975) reported extensive coliforms and *Staphylococci* contamination in *Burfi* and *Pedha* samples in Allahabad market. The extent of contamination was higher in latter than the former article. Singh et al. (1975) examined the microbial quality of indigenous concentrated milk products such as *Khoya*, *Khurchan*, *Pedha* and *Rabbri* from Agra market and reported that *Rabbri* had the highest microbial counts.

Dwarkanath and Srikanta (1977) studied the microbiological quality of fresh, stored and sugar syrup soaked sweets procured from the market of Mysore. Fresh *Dudh Pedha*, stored *Dudh Burfi* and all samples of sweets soaked in sugar syrup excepting *Jalebi* and *Jangree* were found contaminated with coliforms. Only stored samples of *Dudh Pedha* and *Dudh Burfi* indicated the presence of *S. aureus* and *Salmonella*, respectively.

Most Indian food are well heated, but there may be lengthy periods of time between cooking and eating. Some foods may be heated again immediately before eating. The growth which may have occurred earlier will then be killed. For example, *Samosas* were eaten at a morning tea break by a team of workers in Clinical Microbiology Laboratory in Ludhiana (Hobbs, 1982). Suspecting that they were not fresh, a sample was stained by Gram's method; enormous numbers of Gram-positive bacilli resembling *C. perfringens* were seen, but anaerobic and aerobic cultures were sterile and the ten persons who ate the food remained well.
2.1.5 Fruits and vegetables

The main routes of transmission of organisms of public health significance in plant and plant products are via soil, water, fertilizer, direct and indirect faecal contamination and poor hygiene in handling food.

Generally the fruit and fruit juices are sufficiently acidic to inhibit the growth of the organisms. Also fruits are produced above ground and are less likely to be contaminated by polluted water used for irrigation or by direct faecal contamination from Farm Yard Manure except the bird droppings and insect excretion. Nevertheless, diarrhoeal outbreaks after the consumption of fruits and fruit products are on record.

Velaudapillai et al. (1969) surveyed fruit and vegetable samples for Salmonella, Shigella and EPEC. Only one out of 392 fresh fruit samples and 1 to 3% of the fresh vegetable samples yielded enteropathogenic E. coli. Food handlers and irrigation water were considered to be possible sources of contamination.

In 1974, an outbreak of S. typhimurium gastroenteritis occurred in the USA following the consumption of non-sterile apple juice (Anonymous, 1975), the organism was isolated from 154 of 296 patients, 6 of 30 employees and from two bottles of apple cider. Goverd et al. (1979) found that S. typhimurium can survive in apple juices at pH 3-6 or even higher, for a period of 30 days. These workers suggested that apple juice should be produced below 4.5 pH and pasteurized before sale.
Human faeces can contaminate foods in more than one way, such as when 1) added as Farm Yard Manure in fields or kitchen gardens, ii) in sewage waste or water used to irrigate crops and iii) in contaminated water used to wash fruit and vegetables or for growing shellfish.

Roberts et al. (1982) examined 100 samples of various types of vegetables and found that the plate count ranged from less than $10^2$ to more than $10^9$ organisms/g, the lowest being in aubergine, white cabbage and tomato and the highest in bean shoots, cress, spring onions and watercress. Only 14 samples contained *E. coli*, the highest level was 460/g in a sample of bean shoot. *S. dublin* was isolated from only one of the 100 samples examined. *B. cereus* and *C. perfringens* were isolated from one-third of the samples.

2.2 Pathogenesis of bacterial diarrhoea

Levine et al. (1983) classified human enteric pathogens on the basis of the mode of action. The virulence factors recognised were adhesins or colonizing factor antigens (CFA), enterotoxins and mucosal invasion. Some bacteria produce multiple virulence factors.

*C. jejuni* (Skirrow, 1977) and *Y. enterocolitica* species (Bottone, 1977) invade the mucosa and penetrate the lamina propria and mesenteric lymph nodes. These bacteria can cause bacteraemia and may also produce enterotoxin.

Enterotoxigenic bacteria such as *V. cholerae* and *E. coli* (ETEC) elaborate enterotoxins after adherence to the intestinal mucosa but without causing any morphological damage (DuPont et al. 1971; Carpenter, 1972; Norris, 1974; Levine, 1981). Mucosal adheren
also occurs in enteropathogenic *E. coli* (EPEC). The EPEC cause focal disruption and dissolution of brush borders at the site of attachment to the intestinal mucosa but the damage does not extend deeper than the brush border (Edelman and Levine, 1983). Some EPEC produce a cytotoxin similar to Shiga toxin, which may be called verotoxin (O'Brien & Laveck, 1983).

Invasion of the intestinal mucosa and proliferation within the epithelium occur with *Shigella* and closely related enteroinvasive *E. coli* (EIEC), which may penetrate the lamina propria. Occasionally mesenteric lymph nodes are infected although without bacteremia, *S. typhi* and *S. paratyphi* are enteroinvasive, they penetrate the lamina propria, reach the mesenteric lymph nodes and then enter the blood stream via the thoracic duct (Formal et al, 1983).

*Clostridium difficile* and *Bacillus cereus* produce cytotoxic enterotoxins responsible for inflammation and haemorrhage in the intestinal mucosa without penetration by the micro-organisms (Stephen et al, 1984).

2.3 Prevalence of etiological agents in acute diarrhoea

Lewis et al. (1979) in a study of 152 children with acute diarrhoea identified various enteropathogens namely Rotavirus (50.6%); Adenovirus (4.5%); Enterovirus (7.8%); Astrovirus (0.6%); Coronavirus (0.6%); Calicivirus (1.3%); Salmonella species (5.9%); *Shigella* sp. (5.2%); EPEC (0.6%) and *Campylobacter* (3.2%). Jewkes et al. (1981) examined 73 adults with acute diarrhoea and found
Salmonella (19.2%), Campylobacter (17.8%), Sh. flexneri (6.8%), Cl. difficile (5.5%), G. lamblia (4.1%), rotavirus (4.1%), Norwalk type virus (2.7%) and Candida albicans (1.4%). According to Howard (1986), the major causative agents are E. coli (40.70%), Shigella (5-20%), Campylobacter, rotavirus, parvovirus, G. lamblia and other unidentified agents (25-30%).

Sarkar et al. (1980) studied 2018 diarrhoeal infants and children up to the age of 12 years in Delhi. Out of 1326 patients below 2 years of age, 66 were positive for V. cholerae, 6 for NAG vibrio, 14 for Salmonella, 11 for Citrobacter, 490 for E. coli (pure culture) and 160 for Ps. pyocyanae (pure culture).

Ganguly et al. (1980) investigated diarrhoeal stool samples of children and adults in Calcutta. In patients below 2 years of age, the microorganisms isolated were V. cholerae (32%), EPEC (8.4%), Non-EPEC (52.6%) and P. aeruginosa (3.5%). Klebsiella and V. parahaemolyticus were isolated from one patient each. Among adult patients, V. cholerae (26%), VPH (24%), EPEC (14%), Non-EPEC (32%), S. flexneri (2%), P. aeruginosa (10%) and NAG vibrio (2%) were isolated.

In the USA Pickering et al. (1981) investigated the occurrence, cause and transmission of gastroenteritis among children, staff and family members of 20 children's Day-care centres. Nine centres had 15 outbreaks of diarrhoea involving 195 persons. Shigella was detected in five outbreaks, rotavirus in two, Giardia in one and multiple enteropathogens were identified in the remaining seven cases. Rotavirus and Giardia occurred only
in children below 3 years of age while *Shigella* in all ages. Thirty four family members (11%) developed diarrhoea associated with gastroenteritis in children. Secondary attack rates of diarrhoea in families according to organisms identified were *Shigella* (26%), rotavirus (15%) and *G. lamblia* (17%).

Stintzing *et al.* (1981) studied the seasonal occurrence of enterotoxigenic bacteria and rotavirus in paediatric diarrhoea in Addis Ababa. Enterotoxigenic bacteria were isolated in 12.3% of the patients and 4.5% of controls, with a corresponding incidence of rotavirus in 27.8% and 8%, and parasites in 6.8% and 1%. Two peak occurrence were observed i.e. first in August (32.6%) and second in January (19.2%). Also two peaks for rotavirus, were seen in June (42.7%) and November (36.4%). The isolation rate of parasites was not consistent during the year.

Sircar *et al.* (1984) conducted a longitudinal study of diarrhoeal disease in a group of 383 children below 5 years of age in two typical slums of Calcutta. The incidence was higher in patients below two years of age and declined progressively with advancing age. Enteropathogens identified in diarrhoeal stool samples from 13.7% of children included EPEC (5.8%), rotavirus (5.6%), *Shigella* (3.5%), *Salmonella* (1%), *E. histolytica* (0.9%), *G. lamblia* (0.4%), *V. cholerae* (0.6%) and *V. parahaemolyticus* (0.3%).

Mata *et al.* (1984) in a retrospective analysis of agents in acute and chronic diarrhoea observed, *Giardia* and *Shigella* most frequent (about 20%) each) followed by rotavirus and *E. histolytica*
(about 10%) each. Although 59.6% of the patients harboured at least one potential pathogen, Shigella and Giardia appeared singly in only 10 to 11% of instances. Isolations from cases were 41, 14 and 3.7 per cent with single, double and triple pathogens, respectively.

Sen et al. (1984) in a study of 240 diarrhoeal patients of all ages and both sexes from Calcutta reported Vibrio eltor (32.5%), ETEC (12.1%), EPEC (10.4%), rotavirus (8.8%), V. parahaemolyticus (7.5%), C. jejuni (6.7%), Shigella species (5%) and Salmonella species (0.4%). Sambasiva Rao and Vijayalakshmi (1985) examined 2484 patients of all age groups for gastroenteritis at Pondicherry and discovered 13.5% conventional pathogenic bacteria. S. typhimurium constituted the largest single pathogen (61.01%), Sh. dysenteriae type 1 (15.2%) and Vibrio eltor (4.2%).

Sen et al. (1985) in another study from Calcutta reported that out of 88 children below 5 years of age, 18.7% had diarrhoea due to a single or mixed infection including V. cholerae (22%), V. parahaemolyticus (1.2%), ETEC (8.5%), rotavirus (14.6%), EPEC (15.9%), Shigella species (2.4%), C. jejuni (4.9%), G. lamblia (2.4%), V. cholerae and C. jejuni (7.3%), V. cholerae and rotavirus (1.2%), C. jejuni and Sh. sonnei (1.2%). While 133 patients above 5 years of age showed V. cholerae (36%), V. parahaemolyticus (10.5%), ETEC (15.8%), rotavirus (1.5%), Shigella species (4.5%), C. jejuni (1.5%), E. histolytica (1.5%), G. lamblia (1.5%), V. cholerae and C. jejuni (2.8%), V. cholerae and rotavirus (2.3%), V. cholerae and V. parahaemolyticus (0.8%), KPEC and Sh. flexneri (0.8%) and E. histolytica and G. lamblia (0.8%).
Bhat et al. (1985) studied 379 children below 5 years of age with acute diarrhoea and 363 healthy controls in Bangalore. They observed 72.3% rate of isolation for rotavirus and bacterial enteropathogens. Among the different age groups of 6 and 7-12 months, 1, 2, 3 and 5 years, rotavirus was 2.7, 21.1 and 8.4% among patients up to 2 years of age. Shigella was found in all age groups (4.1, 15.8, 32.5, 43.3 and 33.3%), respectively. Salmonella was found in first 3 groups (22.9, 9.7 and 9.6%), EPEC (14.9, 7.3, 13.3, 3.3 and 7.4%), ETEC (6.7, 6.0, 6.1, 10.0 and 18.5%), C. jejuni only in first 4 groups (1.3, 3.0, 6.0 and 3.3%) and mixed isolations were found in (2.7, 10.9, 8.4, 10.0 and 14.8%) cases. Among the control group, 5 were positive for rotavirus, 2 for S. flexneri and 11 for EPEC. Rotavirus diarrhoea was prevalent throughout the study period.

Huq et al. (1985) studied infantile diarrhoea in Riyadh and isolated diarrhoeal pathogens from 30.6% of the children. Rotavirus was found in 16.7%, EPEC in 5.5%, ETEC in 4.6% and Shigella species in 3.7% of the patients. Rotavirus predominated in the age groups of 1 month to 2 years.

Recently, Choudhary et al. (1986) investigated 105 stool samples from diarrhoeal patients in Ranchi (Bihar). The various enteropathogens found were C. perfringens type A (32.4%), S. aureus (11.4%), E. coli (7.6%), Klebsiella sp. (6.7%), Salmonella (5.7%), B. cereus (4.8%), P. aeruginosa (3.8%) and A. hydrophila (2.9%).

Prevalence rates in various studies would reflect differences in laboratory methods used and also the geographical, ecological and socio-economic conditions of human populations.
Comprehensive laboratory investigations reveal potential pathogens in 60 to 70% of community diarrhoeas (Howard, 1986) but many cases remain of unknown etiology and are still designated as "nonspecific diarrhoea".

2.3.1 Escherichia coli

It has been known for many decades that E. coli as a group can cause diarrhoeal disease (Bray, 1945). Recently, it has been classified into four main categories (Levine, 1987) on the basis of virulence, interactions with the intestinal mucosa, clinical syndrome, epidemiology and O:H serotypes (Table 2.1). These categories are (1) enterotoxigenic E. coli (ETEC), (2) enteropathogenic E. coli (EPEC), (3) enteroinvasive E. coli (EIEC) and (4) enterohaemorrhagic E. coli (EHEC). A little known fifth category is enteroadherent E. coli (EAEC).

2.3.1.1 Enterotoxigenic E. coli (ETEC)

ETEC came to prominence in the late 1960s and early 1970s as a cause of watery diarrhoea in humans in Calcutta (Gorbach et al., 1971; Sack et al., 1971). Gorbach and Khurana (1972) found an exceptionally high incidence of ETEC among diarrhoeal children in Chicago. Only a few cases of ETEC induced diarrhoea have been identified in Canada (Gurwith and Williams, 1977). In Britain, ETEC has been implicated in a few outbreaks of diarrhoea in hospitals (Gross et al., 1976; Rowe et al., 1977; Scotland et al., 1977). Outbreaks of ETEC associated diarrhoea
Table 2.1 Comparison of different categories* of diarrhoeagenic *E. coli*

<table>
<thead>
<tr>
<th>Category</th>
<th>Common serotypes</th>
<th>Clinical syndrome</th>
<th>Pathogenesis</th>
<th>Plasmid involvement (size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETEC</td>
<td>06, 08, 015, 020, 025, 027, 063, 078, 080, 085, 0115, 0128, 0139, 0148, 0153, 0159, 0167</td>
<td>Watery diarrhoea</td>
<td>Attack by means of CFA's</td>
<td>LT or ST or both</td>
</tr>
<tr>
<td>EPEC</td>
<td>026, 055, 086, 0111, 0119, 0125, 0126, 0127, 0128, 0142, 018, 044, 0112, 0114</td>
<td>Acute and chronic infantile diarrhoea</td>
<td>Adhere tightly to enterocytes and leads to loss of microvilli (seen on electron microscopy)</td>
<td>Shigella toxin</td>
</tr>
<tr>
<td>EIEC</td>
<td>028, 029, 0124, 0134, 0143, 0144, 0152, 0164, 0167</td>
<td>Dysentery diarrhoea</td>
<td>Invade and multiply within enterocytes</td>
<td>Shigella toxin</td>
</tr>
<tr>
<td>EHEC</td>
<td>0157, 026, 0111</td>
<td>Haemorrhagic Do not invade colitis</td>
<td>Shiga toxin</td>
<td>70 Mdalton</td>
</tr>
</tbody>
</table>

* Modified from Levine and Edelman (1984)
have also occurred in USA (Rosenberg et al., 1977), Japan (Kudoh et al., 1977) and Sweden (Back et al., 1978).

ETEC is now considered responsible for 20-30% episodes of acute endemic diarrhoea in children up to two years of age and traveller's diarrhoea in tropical or subtropical countries (Sack, 1978).

Pickering et al. (1978) conducted a two year study of 255 children with diarrhoea and 112 age matched control without diarrhoea in Houston. Potential enteropathogens were isolated from faeces of 58% patients and 5% of controls. However, ETEC were isolated in only 9% patients and 2% controls.

Except in an occasional nursery outbreak, ETEC are an uncommon cause of infant diarrhoea in USA and Canada (Edelman and Levine, 1980). ETEC is one of the main bacterial cause of dehydration in infantile diarrhoea in developing countries (Black et al., 1981). In a study from Delhi, Kanwar et al. (1983) reported 27.4% of diarrhoeal E.coli strains, positive for LT or ST enterotoxin. Gowal et al. (1984) in comprehensive study of 315 E.coli strains isolated from diarrhoeal cases, found 22.2% ETEC strains, without any overlap with EPEC. Incidence of traveller's diarrhoea reported by various workers are shown in Table 2.2

2.3.1.1.1 ETEC serotypes

Strains of ETEC appear to have a limited number of 'O' antigens, with 06, 08, 015, 025 and 078 isolated most commonly (Orskov et al., 1976). Several reports suggest that the production of enterotoxin by E.coli is serotype specific (Merson et al., 1979)
<table>
<thead>
<tr>
<th>Place</th>
<th>No. of persons at risk</th>
<th>% of diarrhoeal attack rate</th>
<th>% of diarrhoea due to ETEC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia, Africa</td>
<td>28</td>
<td>39</td>
<td>36</td>
<td>Shore et al. (1974)</td>
</tr>
<tr>
<td>Mexico</td>
<td>133</td>
<td>29</td>
<td>45</td>
<td>Gorbach et al. (1975)</td>
</tr>
<tr>
<td>Mexico</td>
<td>121</td>
<td>49</td>
<td>38</td>
<td>Merson et al. (1976)</td>
</tr>
<tr>
<td>Mexico</td>
<td>67</td>
<td>55</td>
<td>75</td>
<td>DuPont et al. (1976)</td>
</tr>
<tr>
<td>Kenya</td>
<td>39</td>
<td>36</td>
<td>50</td>
<td>Sack et al. (1978)</td>
</tr>
<tr>
<td>Thailand</td>
<td>25</td>
<td>57</td>
<td>45</td>
<td>Echevarria et al. (1981)</td>
</tr>
<tr>
<td>Honduras</td>
<td>22</td>
<td></td>
<td></td>
<td>Santosham et al. (1981)</td>
</tr>
</tbody>
</table>
which is useful in screening with polyvalent antisera. Hence, the more complex and time consuming methods for toxin tests could be avoided (Merson et al., 1980). When a collection of ETEC from diverse geographic areas were sero typed, a small number of O:H serotypes occurred repeatedly throughout the world (Black et al., 1981; Schevzeria et al., 1982; Levine et al., 1983). Yet the pattern of prevalent serogroups varied significantly in different parts of the world (Berry et al., 1982). Thus restricting the application of this method exclusively for identification of ETEC, the occurrence of various ETEC serotypes reported in several studies by different workers is shown in Table 2.3.

2.3.1.1.2 ETEC Enterotoxins

ETEC produce noninvasive, plasmid mediated heat-labile (LT \textsubscript{pig} and LT \textsubscript{human}) and heat-stable (ST \textsubscript{pig} and ST \textsubscript{mouse}) cytotoxic enterotoxins. The toxins have differential biological activities and immunologically distinct properties (Aldridge et al., 1978; Gyles, 1979; Takeda et al., 1983; Gemmell, 1984; Betley et al., 1986). Properties of LT and ST enterotoxins are shown in Table 2.4 and methods of detection of LT and ST in Table 2.5.

ETEC have been shown to contain plasmids encoding genes for LT, ST or both (Smith and Halls, 1968). Plasmids carrying ST but not LT genes are heterogeneous (MW 2.1 to 80 x 10\textsuperscript{7}) (Gyles et al., 1974), whereas plasmid that encode both LT and ST genes (MW 55-51 x 10\textsuperscript{6}) tend to be highly related to each other and to other plasmids (Gyles et al., 1974; So et al., 1976). The genes for enterotoxins and CFA/I and CFA/II are usually on the same plasmid (Penaranda et al., 1983; Smith et al., 1984).
<table>
<thead>
<tr>
<th>Diarrhoeal patient</th>
<th>Place</th>
<th>ETEC serotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Calcutta</td>
<td>06,015,025,078,0126</td>
<td>Gorbach et al. (1971)</td>
</tr>
<tr>
<td>Adults</td>
<td>Calcutta</td>
<td>016:H39</td>
<td>Sack et al. (1971)</td>
</tr>
<tr>
<td>Neonates</td>
<td>England</td>
<td>026</td>
<td>Skerman et al. (1972)</td>
</tr>
<tr>
<td>Adults</td>
<td>Kenya, Brazil</td>
<td>027:H20</td>
<td>Sherr et al. (1973)</td>
</tr>
<tr>
<td>Adults</td>
<td>Dacca</td>
<td>078</td>
<td>Evans et al. (1973)</td>
</tr>
<tr>
<td>Children</td>
<td>Arizona</td>
<td>0142</td>
<td>Evans and Evans (1977)</td>
</tr>
<tr>
<td>Children and adults</td>
<td>Arizona</td>
<td>06,025,0109:H1,015,064:H</td>
<td>Sack (1975)</td>
</tr>
<tr>
<td>Children and adults</td>
<td>Sweden</td>
<td>06,08,025,026,048,088,0114,014</td>
<td>Back et al. (1980)</td>
</tr>
<tr>
<td>NM</td>
<td>Dacca</td>
<td>06,08,015,020:K,028:K79,025,027,063,078,0115,0148,0159</td>
<td>Merson et al. (1990)</td>
</tr>
<tr>
<td>Infant and children</td>
<td>Chandigarh</td>
<td>029,017,076,060,033,078,075,01,036,068,08,078,05</td>
<td>Panhotra et al. (1981)</td>
</tr>
<tr>
<td>Children and adults</td>
<td>Delhi</td>
<td>086,0111,0127,0128,0142</td>
<td>Ramachandran and Varghese (1984)</td>
</tr>
<tr>
<td>Children and Infant</td>
<td>Kasauli</td>
<td>05,017,025,037,055,060</td>
<td>Gowal et al. (1984)</td>
</tr>
</tbody>
</table>

**NM** = Not mentioned
Table 2.4 Properties of ETEC LT and ST enterotoxins*

<table>
<thead>
<tr>
<th>Type of toxin</th>
<th>MW</th>
<th>Subunits</th>
<th>Mode of action and other mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT&lt;sub&gt;human&lt;/sub&gt;</td>
<td>86,000</td>
<td>1xA (MW 25,500)</td>
<td>i) Activates adenylate cyclase after binding to specific high-affinity protein receptor and is known to cause a prolonged diarrhoea but lags in initiating the disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5xB (MW 11,500)</td>
<td></td>
</tr>
<tr>
<td>LT&lt;sub&gt;pig&lt;/sub&gt;</td>
<td>86,000</td>
<td>1xA (MW 25,500)</td>
<td>ii) Antigenic and has been used in a purified form for production of immunoassays</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5xB (MW 11,500)</td>
<td>iii) Causes fluid accumulation in 18 hr rabbit ileal loops</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>iv) Chinese Hamster Ovary cells (CHO) elongate morphologically in the presence of LT, whereas Y. adrenal cells round up</td>
</tr>
<tr>
<td>ST&lt;sub&gt;mouse&lt;/sub&gt; (ST&lt;sub&gt;a&lt;/sub&gt;)</td>
<td>2000</td>
<td>Single peptide</td>
<td>v) Resembles cholera toxin in its mode of action</td>
</tr>
<tr>
<td>ST&lt;sub&gt;pig&lt;/sub&gt; (ST&lt;sub&gt;b&lt;/sub&gt;)</td>
<td>5000</td>
<td>Single peptide</td>
<td>vi) Binds to GM1 gangliosides and glycoprotein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vii) It is cytotoxic and is not cytotoxic to cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>viii) Gives skin reaction in 18 hr rabbit skin permeability assay</td>
</tr>
</tbody>
</table>

Table 2.5 Assay for detection of LT and ST enterotoxins

<table>
<thead>
<tr>
<th>Toxin type</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT</td>
<td>i) Rabbit ileal loop (18 hr)</td>
<td>De and Chatterjee (1953)</td>
</tr>
<tr>
<td></td>
<td>ii) Rabbit skin permeability factor (18 hr)</td>
<td>Evans et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>iii) Chinese Hamaster-Ovary (CHO) cells assay</td>
<td>Guerrant et al. (1974)</td>
</tr>
<tr>
<td></td>
<td>iv) Y. mouse adrenal cells assay</td>
<td>Sack and Sack (1975)</td>
</tr>
<tr>
<td></td>
<td>v) ELISA</td>
<td>Yolken et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>vi) Radioimmunoassay (RIA)</td>
<td>Greenberg et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>vii) GM₁ - ELISA</td>
<td>Svennerholm and Holmgren (1978)</td>
</tr>
<tr>
<td></td>
<td>viii) DNA hybridization</td>
<td>Moseley et al. (1980)</td>
</tr>
<tr>
<td></td>
<td>ix) Biken test</td>
<td>Honda et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>x) Coagglutination test</td>
<td>Brill et al. (1979)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Ronnberg and Wadstrom (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Panigrahi et al. (1985)</td>
</tr>
<tr>
<td>STₐ</td>
<td>i) Rabbit ileal loop (6 hr)</td>
<td>Evans et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>ii) Suckling mouse assay</td>
<td>Giannella (1976)</td>
</tr>
<tr>
<td></td>
<td>(4 hr) (1-3 day old)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iii) Radioimmunoassay (RIA)</td>
<td>Gianella et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>iv) ST ELISA</td>
<td>a) Ronnberg et al. (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) De Mol et al. (1985)</td>
</tr>
<tr>
<td>ST₉</td>
<td>i) Weaned piglet (6 hr)</td>
<td>Olsson and Soderlind (1980)</td>
</tr>
</tbody>
</table>
ETEC frequently contain antibiotic resistance markers that can be cotransferred with ST genes (Gross et al., 1976; Echeverria et al., 1978; Smith et al., 1979). The first naturally occurring plasmid reported to contain genes for ST, LT, a conjugal transfer system and antibiotic resistance was PCG 86 (Jiwa, 1981).

*E. coli* LT and ST plasmids have been transferred by conjugation to *V. cholerae* and members of the Enterobacteriaceae (Smith and Halls, 1968; Neill et al., 1983; Takeda et al., 1983; Yamamoto et al., 1984). Klebsiella, Enterobacter and Citrobacter species that contained the ST encoding plasmid caused fluid accumulation in infant mouse and rabbit ileal loop tests (Yamamoto et al., 1984).

*In vitro* study of LT production by ETEC depends upon the incubation time, temperature and pH of the medium. De et al. (1956) suggested optimum pH 8.4 for maximum enterotoxin production. Smith and Halls (1967) found that greatest amount of extracellular enterotoxin was present after 14 hr to 3 days of incubation. The amount then gradually decreased and was very little after 8 days.

*E. coli* enterotoxin can be produced in a variety of media and one of the earliest to be tried was syncase broth (Finkelstein et al., 1966) because of its marked success in cholera enterotoxin production. This semisynthetic media allowed the organism to elaborate both LT and ST. Lariviere et al. (1973) successfully used peptone dialysate broth supplemented with glucose. However, Evans et al. (1973) used semisynthetic media containing casaminoacids and yeast extract for enterotoxin production. Dorner et al. (1976) used peptone water medium containing trypticase peptone, phyton peptone and sodium chloride supplemented with glucose for
enterotoxin production. Media composition can affect LT production in the laboratory. The aminoacids methionine and lysine with either asparatate or glutamate stimulate LT synthesis two to three fold (Gilligan and Robertson, 1979). LT synthesis is highest in the presence of catabolic repressible sugars (Gilligan and Robertson, 1979).

Sublethal concentration of lincomycin and tetracycline stimulate LT synthesis, although other protein synthesis inhibitors have little effect on LT synthesis (Yoh et al., 1983).

Addition of zinc to complex media increases LT production 1.4 to 2.2 fold and the inclusion of a zinc-binding compound blocks the effect of added zinc (Sugarman and Epps, 1984).

Finkelstein and Punyashthiti (1974) reported maximal enterotoxin production by *E. coli* 399 ts (015:H11) when incubated at 35°C for 20 hr with aeration and stirring. Enterotoxin appears in the medium during the log phase and can be detected in as little as 8 hr. Peak concentration was reached in approximately 24 hr followed with plateau over the next few days.

Evans et al. (1974) reported that exposure of intact *E. coli* strain H-10407 Cells to polymyxin B resulted in the rapid release of heat labile enterotoxin. This polymyxin released enterotoxin was found to possess the same biological activities as those associated with heat labile enterotoxin naturally released into broth culture by ETEC.

Crude enterotoxin preparation can be obtained from culture by centrifugation, dialysis of supernatant and lyophilization or concentration by membrane filtration. Lyophilized LT are stable at
refrigeration or room temperature for at least 6 months. Liquid LT preparation may be stored at 4°C, although some loss of activity occurs within a period of weeks (Sack, 1975).

Soderlind et al. (1974) achieved partial purification of heat labile enterotoxin of E. coli strain Ps (0141) by negative absorption on CM Sephadex C-25 and QAE - Sephadex A-25. The enterotoxin was subjected to isoelectric focusing at 4 to 5 pH range with a recovery of 10-20%. The molecular weight estimated from chromatography on Agrose and Biogel A - 0.5 was about one million. The partially purified enterotoxin was devoid of greater enzymatic and hemolytic activities.

Dafni and Robbins (1976) purified heat labile enterotoxin from E. coli 078 H11 by affinity chromatography with antiserum to V. cholerae toxins. Elution of the retained material with 3 M KCNS yielded a nonenterotoxic protein component on sodium dodecyl sulfate gel. After treatment with 2 mercaptoethanol, two protein components were observed. Elution of the affinity column with 5M guanidine yielded an enterotoxin protein that was precipitated with antiserum to V. cholerae. However, Dorner et al. (1976) purified enterotoxin by chromatography and preparative isoelectrophoresis. The resulting product appeared to be pure according to immunoelectrophoresis, disc electrophoresis, ultracentrifugal and immunological criteria. The purified enterotoxin had an apparent MW of the 102,000d and its isoelectric point was 6.9.

Evans et al. (1976) purified polymyxin-B released heat labile enterotoxin of E. coli strain H10407 by using affinity gel
(Affinity Gel-202). This gel possessed a strong and highly specific affinity for the enterotoxin released from intact E. coli cells by polymyxin B.

A partially purified enterotoxin of E. coli strain 711 (P307) by ultrafiltration and precipitation with ammonium sulphate, molecular sieving and an ion exchange column chromatography was achieved by Dorner et al. (1976). The partially purified heat labile toxin behaved like a protein particle of M.W. of 180,000 to 200,000 during molecular sieving and ultracentrifugation. Clements and Finkelstein (1979) developed a purification method for E. coli LT by binding the toxin to terminal galactose residues on agarose beads. Tsuji et al. (1982) observed by GM1-ELISA that toxin of porcine strains (LT_p) belong to separate family from LT_h of human strains.

Characterization of the heat-labile enterotoxin of E. coli was performed by Clements et al. (1982). E. coli were grown in Evans medium (Evans et al., 1973) and the cells were removed by centrifugation. Bacterial cells were extracted with 1M NaCl in phosphate buffer (EXT) and the cells were lysed by sonication (WCL). The cell free supernatant (CFS) was concentrated 50-fold by ultrafiltration. Each of the preparation was then precipitated with 60% saturated (NH_4)_2 SO_4, and the precipitates were harvested and dialyzed against TEAN buffer (Clements and Finkelstein, 1978) and chromatographed on a column of Agarose A-5M. The LT enterotoxin has been isolated in homogenous form with high specific activity from three sources: Cell free supernatant, NaCl extract and whole cell lysates of a ETEC strain. The major portion of the LT remained adherent to
column containing agarose, from which it could be eluted quantitatively in homogenous form by galactose. LT was shown to have 8.0 pH by isoelectric focusing and to have an approximate molecular weight of 91,440 by sedimentation equilibrium determination (Clements et al., 1982).

2.3.1.2 Colonization Factor Antigens (CFA/I and CFA/II) among ETEC strains

The ability of certain ETEC strains to adhere to and colonize in small intestine of humans and animals has been correlated with the presence of specific antigens on the bacterial cell surface. These fimbrial antigens are host specific, protein in nature and capable of agglutinating RBC's from different animal species in the presence of D-mannose (Tixier and Gouet, 1975; Evans et al., 1979).

The human colonization factor antigens I and II (CFA/I and CFA/II) were discovered by Evans et al. (1975 and 1978) and Evans and Evans (1978). According to Levine (1987), the fimbrial colonization factors of human ETEC have certain features in common: (1) They consist of either rigid structures 6-7 nm in diameter or wiry flexible structures 2-3 nm in diameter, (2) They are encoded by plasmids that encodes ST and often LT as well, (3) They consist of protein subunits 14-22 K Da in size (4) In the presence of mannose, many but not all fimbrial colonization factors mediate haemagglutination of certain erythrocytes, (5) CFA's are expressed in cultures grown at 37°C, but not at 18°C and (6) Particular colonization fimbrial are largely restricted to certain O:H serotypes.
CFA/I was found in ETEC serotypes 015, 025, 063, 078 and 0128 (Evans et al., 1975 and 1978). CFA/II was associated with serogroup 06, 08, 080 and 085. ETEC strain possessing CFA/I gave MRHA with RBCs of human, bovine and chicken while CFA/II positive isolates showed MRHA with bovine and chicken erythrocytes (Gaastra and Graaf, 1982).

Cravioto et al. (1982) showed that strains tentatively identified as CFA/II positive on the basis of haemagglutination patterns consisted of three distinct antigens 1, 2 and 3. Smyth, (1982) referred to these antigens as CS1, CS2 and CS3. However, depending on the serotypes and biotype, strains expressing CS1 and CS3, CS2 and CS3 or CS3 only. Thomas et al. (1982) described a new putative colonization factor fimbria in prototype strain E8775. This new CFA was found in ETEC serotypes 025, 0115 and 0167.

Recently, McConnell et al. (1985) reported that E8775, like CFA/II, consists of three distinct antigens. Two of these namely CS4 and CS5 are rigid 6-7 nm fimbriae, whereas CS6 is not. CS4, CS5 and CS6 have been observed in serotypes 027 and 0148. Levine (1987) more recently described a nonhaemagglutinating fimbriae in serotype 0159:H4 strains. Two recent studies from Asia suggest that the proportion of CFA positive ETEC is quite high (Changchawalit et al., 1984; Gotheors et al., 1985) particularly if fresh E. coli isolates are examined.

Evans et al. (1978) prepared a hyperimmune serum against purified CFA/I and cell suspensions of different CFA/I positive strains which were tested for CFA/I production by titration with
The known positive strains were negative when grown on MacConkeys agar. The most efficient medium for detecting CFA/I on E. coli isolates was a peptone agar medium composed of 2% peptone, 0.5% NaCl and 2% agar. Tergitol grown cells were either negative or gave a very low titer. Maximum CFA/I titers were obtained on CFA agar, consisting of 1% casamino acids, 0.15% Yeast extract, 0.005% MgSO₄, 0.0005% MnCl₂ and 2% agar with 7.4 pH. Neither CFA/I nor haemagglutination were produced on ETEC strains grown at 18°C. Agglutinated bacteria can be eluted from the erythrocytes at 37°C and the haemagglutinating property is destroyed by heating the cells at 65°C for 30 min (Gaastra and Graaf, 1982).

Levine et al. (1983) studied the stability of CFA expression among ETEC strain. CFA expression in positive strains remained stable for five subcultures, with only one of nine strains appearing negative. However, by the 10th subculture, three of the nine strains were negative. Out of these three CFA negative strains two belonged to CFA/I and one CFA/II strains. The two strains lost ST as well as CFA/I but retained LT, whereas the CFA/II strain lost both ST and LT in addition to CFA/II.

Faris et al. (1985) reported rapid identification of CFA/I and CFA/II by CoA test. The CoA reagents were prepared using specific CFA/I or CFA/II antisera. It was observed that the CoA test was specific for the respective antigen which neither reacted with type 1 fimbriae nor with F 9 fimbrial antigen.
2.3.1.2 Enteropathogenic E. coli (EPEC)

The term EPEC was coined by Neter et al. (1955) for a collection of known and incriminated serotypes of E. coli. EPEC strains were recently defined at a workshop on EPEC as diarrhoeagenic E. coli belonging to serogroups epidemiologically incriminated as pathogens, but their pathogenesis has not been related with any of the LT or ST enterotoxins and Shigella-like invasiveness (Edelman and Levine, 1983).

EPEC infections are clinically more severe than non-bacterial gastroenteritis or shigellosis (Gurwith et al., 1978). EPEC is common in developing countries. Gastroenteritis caused by EPEC has been reported frequently from Canada (Gurwith and Williams, 1977), South Africa (Robins et al., 1980), Indonesia and Israel (Goldhar et al., 1981). In contrast, at present EPEC infections appear to be relatively uncommon in the United States (Edelman and Levine, 1983).

A workshop on EPEC was held at the National Institutes of Health, Bethesda, Maryland, in September, 1982 (Edelman and Levine, 1983). A brief account of fifty investigators' discussion on epidemiology, diagnosis and pathogenesis of EPEC is given in Table 2.6. The incidence of infantile diarrhoea due to EPEC has been reported from various parts of India by several workers and is summarized in Table 2.7.

In the early 1970s, with the recognition of LT and ST enterotoxins and epithelial cell invasiveness as virulence properties
<table>
<thead>
<tr>
<th>Country</th>
<th>Place</th>
<th>Years</th>
<th>EPEC serotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Israel</td>
<td>1962</td>
<td>MX</td>
<td>Dr. Marc J. Gurwith, Michigan State University, East Lansing</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>1962</td>
<td>0111</td>
<td>Dr. Laneu Sechter, Central Laboratory, Jerusalem</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>1959-1968</td>
<td>026:H11, 055:H5, 011:11, 0111:11</td>
<td>Dr. Frits Orskov, Statens Seruminstitut, Copenhagen</td>
</tr>
<tr>
<td></td>
<td>Denmark</td>
<td>1959-1968</td>
<td>026:H11, 055:H5, 011:11, 0111:11, 0125:H21, 0127:H21</td>
<td>Dr. Luiz R. Trabulsi, Escola Paulista de Medicina, Sao Paulo, Brazil</td>
</tr>
</tbody>
</table>

Common EPEC serotypes implicated with outbreaks of diarrhoea in different countries

Adopted from Edelman and Levine, 1983

NM = Not mentioned
Table 2.7 Enteropathogenic *E. coli* (EPEC) serotypes isolated from diarrhoeal patients by various workers in India

<table>
<thead>
<tr>
<th>Place</th>
<th>Commonest EPEC serotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcutta</td>
<td>026</td>
<td>Banerjee <em>et al.</em> (1957)</td>
</tr>
<tr>
<td>Vellore</td>
<td>055, 026, 0111</td>
<td>Bhat and Myers (1961)</td>
</tr>
<tr>
<td>Pondicherry</td>
<td>026, 055, 0111</td>
<td>Goyal and David (1961)</td>
</tr>
<tr>
<td>Delhi</td>
<td>018, 020, 044, 055, 0111, 0126, 0127, 0128</td>
<td>Prakash (1962)</td>
</tr>
<tr>
<td>Delhi</td>
<td>020, 0128</td>
<td>Prakash (1962a)</td>
</tr>
<tr>
<td>Visakhapatnam</td>
<td>055, 026</td>
<td>Naidu (1962)</td>
</tr>
<tr>
<td>Aurangabad</td>
<td>086, 055, 0127</td>
<td>Sengupta and Sharma (1967)</td>
</tr>
<tr>
<td>Western India</td>
<td>086, 026</td>
<td>Chakraborty (1972)</td>
</tr>
<tr>
<td>Allahabad</td>
<td>026</td>
<td>Pande <em>et al.</em> (1973)</td>
</tr>
<tr>
<td>Lucknow</td>
<td>026, 0112, 0119, 0126</td>
<td>Tewari <em>et al.</em> (1979)</td>
</tr>
<tr>
<td>Ludhiana</td>
<td>04</td>
<td>Pawa and Hobbs (1980)</td>
</tr>
<tr>
<td>Delhi</td>
<td>055, 026</td>
<td>Sarkar <em>et al.</em> (1980)</td>
</tr>
<tr>
<td>Ambajogai</td>
<td>055, 0127, 0124, 020</td>
<td>Fule and Kaundinya (1985)</td>
</tr>
<tr>
<td>Delhi</td>
<td>020, 018, 026, 0119, 0125, 026</td>
<td>Aggarwal and Singh (1987)</td>
</tr>
</tbody>
</table>
of *E. coli* associated with diarrhoea, several workers took up the classical serotype of EPEC for investigations on these properties (Gross *et al.*, 1976; Echevarria *et al.*, 1976; Goldschmidt and DuPont, 1976; Sack, 1976; Gangarosa and Merson, 1977). It was concluded that the "classical" serotypes of EPEC were not pathogenic. Sack (1976) and Gangarosa and Merson (1977) postulated that these strains possessed enterotoxin plasmids when freshly isolated, but lost them during storage. The opinion of a second group of workers was that EPEC represented a separate class of *E. coli* causing diarrhoea by pathogenic mechanisms distinct from LT or ST or epithelial cell invasiveness (Gross *et al.*, 1976; Gurwith *et al.*, 1977).

The isolation of EPEC from some persons without diarrhoea is not unusual. Other bacterial enteropathogens such as *Salmonella* (Aserkoff *et al.*, 1970) and *Shigella* (Khan and Shahidullah, 1980) also caused asymptomatic infections, in several subclinical cases.

Lack of pathogenicity in available animal models also has little importance. *Shigella* was accepted as a pathogen long before tests became available in the 1950s to demonstrate its pathogenicity. For half a century, *Shigella* was accepted as a pathogen because of its higher frequency in isolations from patients with diarrhoea and dysentery than from healthy controls.

EPEC 055, 0111 and 0127 have been isolated 20 times more frequently from cases than controls (Bray, 1945; Bray and Beavan, 1949; Gil and Sangster, 1948; Giles *et al.*, 1949; Taylor *et al.*, 1949).

Levine summarized volunteer studies of adults challenged with classical EPEC of infant diarrhoea (Edelman and Levine, 1983).
Three challenge strains, associated with outbreaks of infant diarrhoea in the UK had been stored up to seven years and were negative for LT, ST and invasiveness. When orally given to adult volunteers in doses of $10^6$ to $10^{10}$ organisms with NaHCO$_3$, two of the three strains caused unequivocal diarrhoea. One volunteer purged 5.6 litre of rice-watery stool. Strains recovered from faecal culture of these volunteers were found negative for LT and ST. In a subsequent study on 0114:H$_2$ EPEC strain isolated from a sporadic case of infant diarrhoea, caused diarrhoea in volunteers when orally fed in doses of $10^8$ to $10^{10}$ organisms.

Polotsky et al. (1977) noted that EPEC strains cause a distinctive ultrastructural histopathological lesion in human intestines which can be seen through electron microscopy. These distinctive lesions involve destruction of the microvilli by EPEC with further evidence of invasion. The same ultrastructural lesion was later described in biopsies from infants with classical EPEC serogroup 0125 and 0119 (Ulshen and Rollo, 1980; Rothbaum et al., 1982). Baldini et al. (1983) reported that 30 of 31 classical EPEC strains possessed a plasmid 60 MD in size that encoded the property of adhesiveness to Hep-2 cells. This adhesive property of EPEC was given the name EPEC adherence factor (EAF). Later, Scaletsky et al. (1984) and Nataro et al. (1985) clearly differentiated this localized adherence to Hep-2 and He La cells from diffused adherence and showed that the former was plasmid-mediated.

Nataro et al. (1985) have cloned a 1 kilobase segment of DNA from the EAF plasmid of strain E 2348/69 and found it to be
highly sensitive and specific DNA probe for detection of EPEC that carry the EAF plasmid. EPEC serotypes that do not possess the EAF plasmid and do not show adherence to Hep-2 cells have also been incriminated by epidemiological and volunteers studies as causes of diarrhoea. These EAF negative EPEC were considered as class II EPEC category (Levine, 1987).

Konowalchuk et al. (1977) identified a cytotoxin produced by EPEC strains, which they called verotoxin (VT). Scotland et al. (1980) reported that 25 of 253 EPEC isolated from infants with diarrhoea produced VT. Some EPEC strains elaborate moderate amount of a cytotoxin identical to the Shiga toxin (O'Brien et al., 1982; Cleary et al., 1985; Marques et al., 1986).

2.3.1.3 Enteroinvasive E. coli (EIEC)

Ogawa et al. (1968) stated that some E. coli strains were capable of mucosal invasion and ulceration resulting in large bowel inflammation. DuPont et al. (1971) described certain strains that caused an invasive, dysenteric type of diarrhoea in volunteers. The common serotypes of EIEC are shown in Table 2.1.

The invasive capacity of both EIEC and Shigella is dependent on the presence of large 140 MD plasmid coding for the production of several outer membrane proteins involved in invasive-ness (Harris et al., 1982). EIEC strains resemble Shigella, being non-motile and non-lactose fermenting with serological cross reactions and pathogenesis (Levine, 1987).
EIEC are detected in vitro by their ability to produce keratoconjunctivitis in guinea-pig's eye (Sereny, 1955) and to invade and multiply within He La cells (DuPont et al., 1971). EIEC can also be diagnosed by serotyping (Toledo and Trabulsii, 1983) by an ELISA that detects the outer membrane proteins associated with invasiveness (Pal et al., 1985) and by DNA probes of genes for invasiveness (Wood et al., 1986).

The high prevalence of sporadic diarrhoea in children (20%) due to EIEC was reported by Guerrant et al. (1975). In prospective studies in North America (Pickering et al., 1978) and in Finland (Maki et al., 1980) involving 375 and 283 children with diarrhoea, only 3 and 1 were found harbouring EIEC in the respective groups.

Very few studies were conducted on the role of EIEC diarrhoea in India. Ganguly (1978) did not detect any EIEC strains from 16 children with diarrhoea in Manipur. Similarly none of the 36 E. coli strains isolated during a diarrhoeal epidemic in Calcutta was enteroinvasive (Ganguly et al., 1980). Sarkar et al. (1980) at Delhi reported 2 EIEC out of 50 E. coli strains isolated from children with diarrhoea. In a study from Chandigarh, Panhotra et al. (1980) found that 94 strains of E. coli grown from 127 children with diarrhoea were noninvasive. Although EIEC causes a similar clinical picture as Shigella, yet these are much less prevalent, being responsible for only 7% of invasive diarrhoea (Saluja, 1984). According to Domah et al. (1985) only 6.2% cases of diarrhoea were found to harbour EIEC.
2.3.1.4 Enterohaemorrhagic \textit{E. coli} (EHEC)

A multistate outbreak of haemorrhagic colitis attributed to an unusual clinical syndrome of diarrhoeal disease implicating \textit{E. coli} serotype 0157:H\textsubscript{7} was investigated by Riley \textit{et al.} (1982). The characteristic symptoms in patients were copious bloody diarrhoea without faecal leukocytes. These differed from the classical dysentery due to \textit{Shigella} or \textit{EIEC}, in which fever and scanty stool with blood, mucus and pus cells are common (DuPont \textit{et al.}, 1971).

\textit{E. coli} 0157:H\textsubscript{7} from persons with haemorrhagic colitis and haemolytic uraemic syndrome elaborate phage-encoded potent cytotoxin active on He La and Vero cells (Johnson \textit{et al.}, 1983; 'O' Brien \textit{et al.}, 1983 and 1984; Smith \textit{et al.}, 1984; Scotland \textit{et al.}, 1985; Noda \textit{et al.}, 1985; Karmali \textit{et al.}, 1986; Strockbine \textit{et al.}, 1986). One of these toxins, Shiga-like toxin 1 or Verotoxin 1, is apparently identical to the potent cytotoxin/neurotoxin/enterotoxin produced by \textit{S. dysenteriae} type 1 (Shiga toxin) (Brown \textit{et al.}, 1982; Eiklid and Olsnes, 1983). It reacts with and can be neutralized by antibody to Shiga toxin. Some strains also elaborate a second potent cytotoxin (Shiga-like toxin 2 or Verotoxin 2) that is not neutralized by antibody to Shiga toxin. (O'Brien \textit{et al.}, 1983; Scotland \textit{et al.}, 1985; Noda \textit{et al.}, 1985; Strockbine \textit{et al.}, 1986). 0157:H\textsubscript{7} strains possess a 60-MD plasmid responsible for virulence (Levine, 1987).

Epidemiological studies on EHEC had not been possible owing to the non-availability of suitable methods for screening large number of stool cultures for 0157:H\textsubscript{7} strains. However, reports on its outbreak are on record. VT-positive strains of serogroup 026,
0111, 0113 and 0157 have been implicated in cases of haemolytic uraemic syndrome (Karmali et al., 1983; Levine, 1987 and Krishnan et al., 1987). These workers contended that EPEC serotype 026:H11 should be classified as EHEC due to the presence of bloody diarrhoea. This serotype usually produce abundant verotoxin (O'Brien and La Veck, 1983). EPEC serotype 026 possesses a 60-MD plasmid (Smith et al., 1983). Krishnan et al. (1987) found that in infants serotype 026 appears to be as frequent as 0157.

The detection of a pathogenic strain of *E. coli* among a multitude of nonpathogenic *E. coli* strains and other lactose fermenting coliforms is a difficult task unless differentiating markers are available. Laboratory diagnosis of haemorrhagic colitis is based on the demonstration of a cytotoxin (Vero or Shiga toxin) produced by *E. coli* which is highly toxic for vero cells (Konowalchuk et al., 1977). Serotyping with specific 'O' and 'H' antisera is a valuable tool for the identification of EHEC (Johnson et al., 1983; Wells et al., 1983; Farmer and Davis, 1985).

Most useful markers for detection of *E. coli* 0157:H7 are the absence of fermentation of sorbitol within 48 hr (Wells et al., 1983; Harris et al., 1985; March and Ratnam, 1986), a negative beta glucouronidase reaction and positive raffanose fermentation. The use of MacConkey agar medium containing sorbitol instead of lactose has been well documented. Two of the common EPEC serotypes, 0111:B5 and 055:B5, failed to ferment sorbitol, whereas 93 to 95% of all *E. coli* are sorbitol fermenters (Krishnan et al., 1987).
Laboratory diagnosis of EHEC has been difficult since it requires tissue culture. It is slow and cumbersome. The Verotoxin may be detected from stool filtrate and is very specific and sensitive (Pai et al., 1984; Karmali et al., 1985). The demonstration of toxin production by isolated colonies of E. coli or even from mixed culture, although highly specific, is less sensitive than stool filtrates (Johanson et al., 1983; Karmali et al., 1983 & 1985). Tests for FVT and VTEC take from 2 to 5 days to complete, but can detect all serotypes of E. coli, capable of producing Verotoxin.

2.3.1.5 Enteroadherent E. coli (EAEC)

Preliminary evidence suggests that EAEC are capable of causing diarrhoeal disease and do not fit into the other four categories of E. coli. A recent study in Mexico has shown that E. coli which adhered to Hep-2 cells did not produce LT or ST, did not belong to known EPEC serogroups, but were significantly associated with diarrhoeal episodes (Mathewson et al., 1985). These EAEC were found in 14.9% of 188 patients with diarrhoea and in 7.6% of travellers without diarrhoea. It is feasible that these E. coli represent another group of bacteria which cause diarrhoea by a previously unrecognised and inadequately understood mechanism (Levine, 1987).

2.3.1.6 Drug resistance in E. coli

R-plasmids responsible for transfer of drug resistance in E. coli were demonstrated by various workers (Panse and Wadhwa, 1976; Datta et al., 1980; Agarwal et al., 1981; Rangnekar et al., 1982).
The association between plasmid mediated antibiotic resistance and enterotoxin production was demonstrated in a few studies (Gyles et al., 1977; Echeverria et al., 1978; Panhotra and Agarwal, 1981; Gowal et al., 1984).

Various workers have reported a high incidence of multiple resistant ETEC (Sarkar et al., 1979; DeBoy et al., 1980; Tewari and Agarwal, 1983). Gowal et al. (1985) studied plasmid mediated enterotoxin production and drug resistance in 70 ETEC isolates from infants and children with acute diarrhoea. Sixty one strains were found to be resistant to two or more drugs and 9 were sensitive to all the drugs tested. Most of the strains were resistant to 5 drugs with pattern ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracyclin.

The presence of conjugative plasmid was demonstrated in 34.2% strains. Panigrahi et al. (1985) observed a higher percentage of resistance in non-ETEC strains. While ETEC exhibited resistance to ampicillin, tetracyclin and chloramphenicol (61.5, 57.7 and 42.3% respectively), non-ETEC resistance to these antibiotics was 91.6, 86.5 and 60.8% respectively. Among the ETEC strains, those producing ST alone showed much multiple drug resistance than strains producing LT and ST or LT only.

Multiple drug resistance in EPEC has been observed by various workers (Anderson, 1968; Nakahara et al., 1977). Sarkar et al. (1979) reported a high level of resistance among EPEC isolates from diarrhoea patients in Delhi, the order being ampicillin (94%), streptomycin (92.8%) and chloramphenicol (82.8%).
The multidrug resistance in non-pathogenic *E. coli* is a matter of concern. Agarwal and Singh (1987) studied antibiotic resistance of *E. coli* isolated from patients without diarrhoea. Fifty four percent of *E. coli* strains were resistant to 4 to 6 antibiotics. The resistance pattern of 436 isolates was tetracycline (33.07%), ampicillin (16.9%), chloramphenicol (12.8%), streptomycin (8.7%), kanamycin (2.5%), neomycin (2.3%) and furazolidine (0.7%). There was no indication of any resistance to gentamycin.

### 2.3.2 Salmonella

The *Salmonella* group of organisms are divisible into the enteric species *S. typhi* and *S. paratyphi* A, B and C and those that cause gastroenteritis. The dose required to induce symptoms is far larger than for *Shigella*. On the basis of antigens of *Salmonella*, Kauffmann-white scheme consists of more than 2000 *Salmonella* serotypes (Saxena and Mago, 1983).

The incidence of various serotypes differs greatly among countries. This may be related to the fact that the main reservoir of *Salmonella* are animals and the primary mode of transmission is contaminated foods, usually of animal origin, which are consumed more frequently by some populations than by others (Sharma, 1982).

*Salmonellosis* has been recognized as a world-wide problem both in man and animals. All the recorded serotypes are known to be pathogenic for man. Poultry are especially vulnerable, owing to the contaminated food stuffs and crowded living conditions under the unhygeinic battery system for intensive rearing.
The Salmonella pattern in India is reflected by the strains received from various parts of the country at the National Salmonella and Escherichia Centre at the Central Research Institute, Kasauli, (Agarwal, 1962 and 1963; Nath et al., 1966 and 1970). Basu et al. (1975) in a 16 years study reviewed the prevalence of Salmonella serotypes in India between 1958 to 1973. Out of 8027 strains of Salmonella tested, 3834 strains were from man, 3018 from animals, 839 from sewage and water sources and 336 of unknown origin. A total of 99 serotypes were identified out of which 47 were from man. S. typhi was the commonest in man followed by S. weltevreden and S. paratyphi A. S. typhimurium was the commonest serotype isolated from animals, followed by S. weltevreden and S. anatum. In sewage and other water sources, S. weltevreden was the commonest serotype, followed by S. typhimurium and S. bareilly. Infection due to S. weltevreden in both man and animals increased considerably after 1970.

S. typhimurium is now reported more frequently than any other serotype and has a world wide distribution possibly because of ease of adaptation to both human and animal hosts. The capacity of this pathogen to acquire R factors when animals are with antibiotics has been well documented (Anderson, 1968). It has been reported to be the most common serotypes in England and Wales (Lee, 1974) and USA (Ryder et al., 1976). In India also, it has been implicated in diarrhoeal diseases (Sanyal et al., 1974; Paul et al., 1981; Sharma, 1982; Chaturvedi et al., 1985; Fule and Kaundinya, 1985).
The proficiency of phage typing has enabled the scientists to trace the cause of many outbreaks. During 1982, National Salmonella Phage Typing Centre at Delhi, received 489 strains of \textit{S. typhimurium} from various parts of India (Prakash, 1983). Mostly the strains (90\%) were untypable. The typables belonged to phage 29, 66, 122, 22, 91, 93 and 163, which did not show a definite pattern of occurrence in different regions. Recently an outbreak of \textit{S. typhimurium} phage type 66 occurred in a Pediatric ward at Wardha, Maharashtra (Chaturvedi \textit{et al}., 1985).

More recently serotype pattern of \textit{Salmonella} isolated from different parts of India has been reported by Saxena (1986) and the details are given in Table 2.8.

Some \textit{Salmonella} species have enterotoxic activity (Koupal and Diebel, 1975). Two skin permeability factors isolated from \textit{S. typhimurium} differed in heat stability, time from challenge to reaction and induction of induration in a rabbit skin test (Sandefur and Peterson, 1976). The heat labile permeability factor, which causes delayed reaction and induration, fluid accumulation in rabbit ileal loops and elongation and increased CAMP levels in CHO cells assays was an enterotoxin (Sandefur and Peterson, 1977). These activities were neutralized by cholera antitoxin or ganglioside. \textit{Salmonella} enterotoxin has been purified and is related immunologically and biochemically to CT (Sedlock \textit{et al}., 1978; Finkelstein \textit{et al}., 1983).

The location of the genes for the \textit{Salmonella} enterotoxin has not been determined but there appears to be no phage association (Molina and Peterson, 1980; Houston \textit{et al}., 1982).
Table 2.8  *Salmonella* isolated from India during the year 1986

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>No. of strains</th>
<th>Salmonella serotype</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. gallinarum</td>
<td>153</td>
<td>S. anatum</td>
<td>3</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>637</td>
<td>S. choleraesuis</td>
<td>1</td>
</tr>
<tr>
<td>S. genftenberg</td>
<td>61</td>
<td>S. dublin</td>
<td>613</td>
</tr>
<tr>
<td>S. virchow</td>
<td>9</td>
<td>S. I.4,5,12:b</td>
<td>1</td>
</tr>
<tr>
<td>S. chester</td>
<td>4</td>
<td>S. VI Ferlac*</td>
<td>1</td>
</tr>
<tr>
<td>S. barelly</td>
<td>36</td>
<td>S. litchfield</td>
<td>4</td>
</tr>
<tr>
<td>S. cero</td>
<td>27</td>
<td>S. saintpaul</td>
<td>1</td>
</tr>
<tr>
<td>S. typhi</td>
<td>251</td>
<td>S. stanley</td>
<td>8</td>
</tr>
<tr>
<td>S. paratyphi A</td>
<td>33</td>
<td>S. oranienburg</td>
<td>1</td>
</tr>
<tr>
<td>S. I.4,5,12:---</td>
<td>10</td>
<td>S. infantis</td>
<td>7</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>27</td>
<td>S. guerdhan</td>
<td>5</td>
</tr>
<tr>
<td>S. weltevreden</td>
<td>62</td>
<td>S. bovismorbificans</td>
<td>4</td>
</tr>
<tr>
<td>S. reading</td>
<td>2</td>
<td>S. agona</td>
<td>4</td>
</tr>
<tr>
<td>S. mbandaka</td>
<td>17</td>
<td>S. richmond</td>
<td>2</td>
</tr>
<tr>
<td>S. havana</td>
<td>67</td>
<td>S. VI 6,14:a:1,5*</td>
<td>1</td>
</tr>
<tr>
<td>S. newport</td>
<td>62</td>
<td>S. bronx</td>
<td>1</td>
</tr>
<tr>
<td>S. nchanga</td>
<td>5</td>
<td>S. edinburg</td>
<td>1</td>
</tr>
<tr>
<td>S. chingola</td>
<td>2</td>
<td>S. I.18:---</td>
<td>1</td>
</tr>
<tr>
<td>S. I.4,5,12:1,2</td>
<td>4</td>
<td>S. tennessee</td>
<td>4</td>
</tr>
<tr>
<td>S. I.6,7:---</td>
<td>1</td>
<td>S. derby</td>
<td>2</td>
</tr>
<tr>
<td>S. ohio</td>
<td>16</td>
<td>S. amsterdam</td>
<td>1</td>
</tr>
<tr>
<td>S. hvittingfoss</td>
<td>1</td>
<td>S. bredeney</td>
<td>1</td>
</tr>
<tr>
<td>S. braenderup</td>
<td>1</td>
<td>S. indiana</td>
<td>1</td>
</tr>
<tr>
<td>S. wentworth*</td>
<td>2</td>
<td>S. I.16:IV:---</td>
<td>2</td>
</tr>
<tr>
<td>S. paratyphi B</td>
<td>7</td>
<td>S. I.4,5,12:eh:---</td>
<td>1</td>
</tr>
</tbody>
</table>

* Isolated for the first time in India

Adopted from *Salmonella Surveillance Bulletin No.10* (1986)
Sobeh et al. (1984) investigated 522 strains of various Salmonella serotypes of Indian origin. Enterotoxin production was demonstrated by ileal loop as well as rabbit skin premeability assay in 9.4% of the isolates.

Surveillance of antibiotic resistance is essential as the emergence of drug resistant enteric pathogens is very common in Enterobacteriaceae, plausibly due to R-factors. With a very high incidence of ampicillin, chloramphenicol and streptomycin resistant enteropathogenic E. coli isolated from diarrhoeal children, transfer of resistance by R-factors to enteric pathogens like Salmonella is possible. Therapy with these antibiotics creates a selective pressure on the organisms. Chatterjee and Thawani (1985) studied transferable drug resistance in enteric bacteria isolated from cases of diarrhoea in Calcutta. A search for autotransferable R-plasmids revealed complete or partial transfer of drug resistance in 31.3% strains. The species-wise percentage transfer rate in order of prevalence was Salmonella (60), Klebsiella (40), E. coli (36.3), Citrobacter (33.1), Shigella (33.3), Enterobacter (21.7) and Proteus (15).

During the last one decade, there has been a spurt of multidrug resistant Salmonella strains on Indian horizon. Various studies from different parts of India revealed that Salmonella serotypes have acquired resistance to a large number of antimicrobial agents in common use (Chitkara and Gill, 1976; Panhotra et al., 1980; Paul et al., 1981). Mostly the strains (91.6%) possessed a
common multiple resistance pattern of ampicillin, chloramphenicol, kanamycin, streptomycin and tetracycline (Prakash, 1983). It has been observed in India and abroad as well that after acquiring the R-plasmid, strains of S. typhimurium have become untypable.

Of the 1289 Salmonella strains of 39 serotypes received during 1984 from various parts of India in National Salmonella and Escherichia Centre, C.R.I., Kasauli, 295 were sensitive to all antibiotics, 273 to single, 89 to 2 and 632 to multiple drugs (Saxena, 1984). Drug-wise sensitivity breakup was 54% to tetracycline, 43% to ampicillin and kanamycin, 42% to chloramphenicol, 23% to nalidixic acid, 22% to furazolidine and 8% to gentamycin.

Chaturvedi et al. (1985) while investigating an outbreak of S. typhimurium found that all isolates were sensitive to gentamycin and streptomycin but resistant to chloramphenicol, kanamycin and tetracycline.

Narang et al. (1985) noticed a nosocomial outbreak in the Paediatric ward due to S. senftenberg and found that all the strains were resistant to ampicillin, gentamycin, kanamycin, carbenicillin and nitrofurantoin and sensitive to cephalaridine, cephallexin, polymyxin B, furazolidine and nalidixic acid.

2.3.3 Shigella

Shigella is divided into four groups containing 39 types and serotypes (Levine, 1982). Groups A (S. dysenteriae), B (S. flexneri) and C (S. boydii) contain multiple serotypes, while group D (S. sonnei) consists of a single serotype. Shigella organisms are
capable of causing a spectrum of gastrointestinal illness in man. About 10 of these organisms can cause overt clinical infection in a wellnourished healthy adult (Levine, 1982).

Shigellosis continues to be a major health problem in the developing and developed countries. This is more so in developing countries, where malnutrition and poor hygienic standards prevail, resulting in continuous transmission of the disease with sporadic outbreaks or epidemics of serious proportions. Shigella are host adapted to man and other primates. Their niche is the colon and transmission is by the faecal-oral route. Infected persons whose personal hygiene is poor can contaminate foods during handling. Shigella are among the more difficult enteric bacteria to isolate. Direct plating without delay, use of enrichment broth and in case of delayed processing of samples use of a suitable transport media such as Cary-Blair, are the approaches employed for increased recovery. Shigella are more difficult to isolate from foods than from clinical specimens (Bryan, 1982).

Reports regarding prevalent serotypes of Shigella are available from different parts of India (Table 2.9). In the developed countries, the incidence of S. sonnei accounts for more than 90% of all Shigella infections whereas in developing countries, S. flexneri is more common (Blaser et al., 1983).

Shigellosis is endemic in Bangladesh, S. flexneri being the predominant endemic species, with superimposed epidemics due to S. dysenteriae 1 (Shahid et al., 1987).
Table 2.9 Incidence of *Shigella* serotypes reported by various workers from different parts of India

<table>
<thead>
<tr>
<th>Species</th>
<th>Allahabad</th>
<th>Calcutta</th>
<th>Delhi</th>
<th>Delhi</th>
<th>Calcutta</th>
<th>Chandigarh</th>
<th>South West Coast of India</th>
<th>Chandigarh</th>
<th>Lucknow</th>
<th>Miraj</th>
<th>Ludhiana</th>
<th>Bombay</th>
<th>Srinagar</th>
<th>Bangalore</th>
<th>Lucknow and other places</th>
<th>West Bengal</th>
<th>Delhi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. dysenteriae</em></td>
<td>7.6</td>
<td>53.7</td>
<td>3.3</td>
<td>3.3</td>
<td>3.3</td>
<td>8.0</td>
<td>6.8</td>
<td>9.6</td>
<td>100.0</td>
<td>66.7</td>
<td>38.9</td>
<td>40.0</td>
<td>100.0</td>
<td>20.6</td>
<td>2.4</td>
<td>100.0</td>
<td>20.6</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>15.0</td>
<td>74.0</td>
<td></td>
<td></td>
<td></td>
<td>8.4</td>
<td></td>
<td></td>
<td>100</td>
<td>66.7</td>
<td>38.9</td>
<td></td>
<td>100.0</td>
<td>20.6</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>24.0</td>
<td>56.0</td>
<td>14.0</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. sonnei</em></td>
<td>20.0</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. hadar</em></td>
<td>12.0</td>
<td>57.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. hennii</em></td>
<td>63.6</td>
<td>29.5</td>
<td></td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. indica</em></td>
<td>14.6</td>
<td>78.8</td>
<td>5.4</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. youngae</em></td>
<td>14.6</td>
<td>78.8</td>
<td>5.4</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference:
- Panda and Gupta (1964)
- Sharma *et al.* (1967)
- Natraj *et al.* (1968)
- Abraham *et al.* (1969)
- Sakazaki *et al.* (1971)
- Arya *et al.* (1977)
- Stephen *et al.* (1978)
- Vasudev (1978)
- Khan *et al.* (1979)
- Pawar *et al.* (1979)
- Arora *et al.* (1982)
- Kale *et al.* (1982)
- Panhotra and Desai (1983)
- Sultana and Bhargava (1984)
- Agarwal *et al.* (1984)
- Sen Gupta *et al.* (1986)
- Dudeja *et al.* (1987)
The first report of multiple resistant *Shigella* strain in 1952 from a dysentery patient was from Japan (Akiba *et al.*, 1960). It showed resistance to tetracycline, sulphadiazine, streptomycin and chloramphenicol. Frequency of isolation of multiple resistance gradually increased to very high percentage.

The 1968-1969 outbreak in Mexico and Central America, was due to *S. dysenteriae* 1, which was antibiotic sensitive (Gangarosa *et al.*, 1972). The same strains acquired R-factor mediated resistance to chloramphenicol, streptomycin, tetracycline and sulphonamides and later to ampicillin too (Olarte *et al.*, 1976).

Agarwal *et al.* (1981) from Lucknow and Kale *et al.* (1982) from Bombay have reported 63.9% and 50% multi-resistance respectively in *Shigella* from the children. Arora *et al.* (1982) from Ludhiana, investigated 224 strains of *Shigella* and noticed resistance to gentamycin, kanamycin, furazolidine, neomycin and nalidixic acid as 2.1, 19.2, 12.5, 26.3 and 10.7 per cent respectively.

During the investigation of a dysentery epidemic in Kashmir, 96 strains of *S. dysenteriae* 1 were isolated by Panhotra and Desai (1983). These strains possessed resistance to cotrimoxazole but were sensitive to neomycin, furazolidine and nalidixic acid.

The WHO working group (1983) on antimicrobial resistance, reported a higher prevalence of resistance to ampicillin, chloramphenicol, tetracycline and sulphonamides in developing
countries and attributed it to higher consumption of antibiotics. Another factor is the common availability of these drugs in the market and their use without proper medical advice.

Agarwal et al. (1984) reported the commonest pattern of multiple drug resistance, a combination of four drugs namely ampicillin, chloramphenicol, streptomycin and tetracycline (27.7%) followed by that of chloramphenicol, streptomycin and tetracycline (22.2%). Individually, tetracycline resistance was highest (81.5%) followed by streptomycin (78.3%), chloramphenicol (60.2%) and ampicillin (60.2%). Highest resistance (89.1%) was noticed in \textit{S. dysenteriae} followed by \textit{S. flexneri} (68.5%) and \textit{S. boydii} (60.7%).

In a study from Riyadh, Huq et al. (1985) observed that all Shigella isolates were resistant to tetracycline and streptomycin.

In Bangladesh, Shahid et al. (1985) investigated 431 strains of \textit{S. flexneri} for drug resistance and observed that 10% were resistant to ampicillin and only 3 strains to cotrimoxazole. It is further reported that during January-February, 1987, the resistance was 39% in \textit{S. flexneri} and 78% in \textit{S. dysenteriae} 1 strains to ampicillin (Shahid et al., 1987).

Recent reviews on the enterotoxin/neurotoxin elaborated by 
\textit{Shigella} have been published by Levine (1982) and Keusch et al. (1982). \textit{S. dysenteriae} type 1 elaborate a neurotoxin that causes limb paralysis when inoculated into laboratory animals and is cytotoxic to human cells in tissue culture. Keusch et al. (1982) demonstrated that this \textit{Shigella} toxin stimulated intestinal
secretion (enterotoxin). Other Shigella serotypes also have been reported to produce a similar type of toxin (Levine et al., 1983).

2.3.4 Campylobacter

Campylobacter has become an important diarrhoeal agent in infants and children (Butzler et al., 1973; Skirrow, 1977).

Almost all strains of Campylobacter associated with acute enteritis belong to the group with thermophilic character originally described by King (1957). There are two classifications of Campylobacter namely the French (Veron and Chatelain, 1973) and the American (Smibert, 1974). In the American classification all of these strains were included in a single subspecies (C. fetus biotype jejuni) but in the French and currently accepted classification they are divided into two groups depending upon the ability of the organisms to produce catalase. Catalase negative C. spectorum and C. pubulus are considered non-pathogenic for man. The catalase positive group consists of C. fetus which is now divided into two species i.e. C. jejuni and C. coli. A wide variety of animals both wild and domestic serve as the main reservoir of infection (Skirrow and Benjamin, 1980). Sources of human infection are probably poultry, cattle, sheep, pigs and dogs.

Blaser et al. (1980) described the reservoirs for human Campylobacteriosis. In order to determine whether an endogenous human reservoir was present, the isolation rate from several populations suffering from diarrhoea and asymptomatic adults and children were compared. Isolation of C. fetus biotype...
jejuni in 4.1% of patients with diarrhoea was consistent. Untreated patients excreted the organisms in their faeces for several weeks. No prolonged convalescent carriage was noted in these. Rarely it was recovered from the faeces of asymptomatic individuals and not at all from the vaginal flora of women. The common shedding of \textit{C. fetus} biotype \textit{jejuni} in the faeces of domestic animals especially puppies with diarrhoea might represent significant reservoir for human infection.

Chatterjee (1931) in a study of \textit{Campylobacter} enteritis in Calcutta reported for the first time isolation of \textit{C. fetus} biotype \textit{jejuni} from cases of acute diarrhoea with fever.

Rajan and Mathan (1982) observed the prevalence of \textit{C. fetus} biotype \textit{jejuni} in healthy population in southern India. The faecal culture was positive in 14.8% of healthy population in rural areas. The isolation rate was highest in pre-school children. They suggested that environmental factors were important in the prevalence of these organisms. The role of \textit{Campylobacter} organisms in acute diarrhoeal diseases could not be ascertained until the pathogenic organisms were identified.

Glass et al., (1983) described the epidemiological and clinical features of endemic \textit{C. jejuni} infection in Bangladesh. These were studied in diarrhoeal patients infected with \textit{C. jejuni}, healthy control subjects and village children whose faecal samples were cultured monthly and at each diarrhoeal episodes during a ten months period. The rates of isolation were greatest in infants and declined with increase in age. \textit{C. jejuni} was less frequently isolated from village children with diarrhoea.
Patients having diarrhoea and healthy control subjects had an elevated convalescent phase antibody titer as determined by complement fixation test. Enteric infections with \textit{C. jejuni} were common in Bangladesh but often asymptomatic, although pathogenicity was determined by serologic response in some patients.

Nair \textit{et al.} (1984) reported the occurrence and significance of \textit{C. jejuni} in Calcutta in India. The recovery rate from normal individuals was 4.4\% while 7.7\% in patients with acute diarrhoea. When the two selective media were used in parallel tests, there was no significant increase in detection rate. The incidence in infection was high among pre-school children. During the four seasons its incidence did not show any marked variation. There was high incidence of mixed infections of \textit{C. jejuni} with other known bacterial enteropathogens.

Ayyagari \textit{et al.} (1984) detected \textit{C. coli} among diarrhoeal and non-diarrhoeal children in Chandigarh. Four fold increase in agglutinating antibody titer was seen in children having diarrhoea. In diarrhoeal and non-diarrhoeal groups, \textit{C. coli} was isolated with frequencies of 3.1\% and 3.8\% respectively. All strains belonged to serotype 49, which were typed by Lior method and designated as Lior serotype 35.

Bhattacharya \textit{et al.} (1985) have described the clinical manifestations of \textit{Campylobacter} enteritis in Calcutta. Watery diarrhoea and vomiting were the main complaints in mixed infection and with infections of \textit{V. cholerae} alone. Bloody
diarrhoea and abdominal pain was observed in the patients having *C. jejuni* infection. Severe dehydration was more in mixed infections than in patients with *C. jejuni* or *V. cholerae*.

An enterotoxin from *C. jejuni* exhibited a closer immunological relationship to *E. coli* LT than to CT (Fernandez et al., 1983; Ruiz-Palacios et al., 1983; McCardell et al., 1984; Klipstein and Engert, 1985).

2.3.5 Aeromonas

*Aeromonas* is now considered an important enteric pathogen around the world. Recent studies in Thailand (Pitarangsi et al., 1982), Australia (Gracey et al., 1982), England (Millership et al., 1983), India (Saraswathi and Deodhar, 1986) and Netherlands (Kuijper and Zanen, 1987) have shown that *Aeromonas* can be recovered more frequently from the diarrhoeal patients than the healthy persons.

The incidence of healthy faecal carriers of *Aeromonas* varies, reflecting the number of bacteria contaminating the drinking water. In a study in Thailand, Echeverria et al. (1981) isolated *Aeromonas* from all drinking water jars and 74% of the Canal water in the area. The incidence of healthy carriers was up to 27% in Thai population (Pitarangsi et al., 1982).

Gracey (1986), in a prospective study of more than 1000 diarrhoeal children and equal number of control subjects, showed that enterotoxigenic *A. hydrophila* was associated with 11% diarrhoeal patients against less than 1% in controls.
Saraswathi and Deodhar (1986) isolated enterotoxigenic *A. hydrophila* as the sole of enteropathogen in 1 per cent of patients with acute diarrhoea and no such organism was found in control samples.

The enterotoxigenic activity of Aeromonas isolates has been demonstrated in rabbit ileal loop assay (Wadstrom et al., 1976). In another study, 50 strains of *A. hydrophila* were isolated from man and environment. Annapurna and Sanyal (1977) reported that most of the strains were toxigenic, similar to that of a toxigenic *V. cholerae* strain.

Recently, some workers have split the species *A. hydrophila* into *A. hydrophila*, *A. sobria* and *A. caviae* (Janda et al., 1984; Turnbull et al., 1984). *A. caviae* is mostly found in the environmental samples (Turnbull et al., 1984). Recently, Watson et al. (1985) in a study observed majority of *A. sobria* strains to be invasive type.

Barer et al. (1986) used five simple and rapid tests for classification of Aeromonas species. These tests include a positive oxidase test, beta haemolysis, growth at 42°C, production of gas in glucose and aesculin hydrolysis in addition to acetoin production and fermentation of salicin and arabinose. Eighty per cent of the Aeromonas isolates could be identified with minimal risk of misidentification.

Kuijper and Zanen (1987) in a study from Netherlands isolated Aeromonas from 195 (0.67%) of 28981 faecal samples. Aeromonas isolates were identified as *A. hydrophila* (15%), *A. sobria* (17%) and *A. caviae* (68%). All *A. hydrophila* and
A. sobria isolates produced a cytotoxin (Verotoxin) and 74% also produced a haemolysin to rabbit erythrocytes. Only 1% of A. caviae showed the production of a cytotoxin while none of the strains produced haemolysin. There was a good correlation between clinical symptoms of diarrhoea and isolation of A. hydrophila and A. sobria. Symptoms of diarrhoeal patients with A. caviae were less acute and milder than in patients with A. hydrophila or A. sobria. Of all A. caviae isolates, 55% were found in children under 5 years of age, whereas A. hydrophila and A. sobria occurred equally at all ages.

A. hydrophila produces an enterotoxin that has been separated biochemically and genetically from the cytotoxin and homolysin (Ljungh et al., 1982; Chakraborty et al., 1984; Turnbull et al., 1984). The Aeromonas enterotoxin is distinct from CT, LT and ST (Chakraborty et al., 1984; Timmis et al., 1984).

2.3.6 Plesiomonas shigelloides

P. shigelloides is a gram-negative, facultative anaerobic late lactose fermenting bacillus that reacted with S. sonnei phase 1 antiserum, but was indole positive and motile. P. shigelloides has been isolated from stools of children and adults with diarrhoeal disease. Mostly reports on the isolation of P. shigelloides are from sporadic cases in Japan (Tsukamoto et al., 1978) and India (Sanyal et al., 1975 & 1980). Pitarangsi et al. (1982) found it from patients and controls in about the same frequency.

Chatterjee and Neogy (1972) isolated P. shigelloides and Aeromonas in 8% cases of choleraic diarrhoea in Calcutta. Bhat et al. (1974) reported an epidemic of such diarrhoea in Vellore. In the same
year, Vandepitte et al. (1974) isolated \textit{P. shigelloides} from 41 patients in Zaire.

Sanyal et al. (1975) isolated \textit{P. shigelloides} from four cases and three carriers while investigating an outbreak of cholera in Varansi. Further, while carrying out a study on diarrhoeal disease of children in a hospital and community population, Sanyal et al. (1977) observed that \textit{P. shigelloides} was responsible for 2.5% and 2.3% of diarrhoeal cases in hospitalized patients and community population respectively.

Tsukamoto et al. (1978) reported two epidemics of diarrhoeal disease due to \textit{P. shigelloides}. Pitarangsi et al. (1982) have evaluated the enteropathogenicity of \textit{P. shigelloides} among persons with and without diarrhoea in Thailand.

Recently, Holmberg et al. (1986) reported 31 isolations of \textit{P. shigelloides} from diarrhoeal patients in U.S.A. Most ill persons had self limited diarrhoea with blood and mucus in stool and other clinical findings suggested enteroinvasiveness of the infecting organisms. Infection with \textit{P. shigelloides} was strongly associated with eating uncooked shellfish and with foreign travel, usually to Mexico. A single large molecular weight plasmid was found in 12 to 27 isolates, but it did not appear to be identical with virulence plasmid of \textit{Shigella} or EIEC (Holmberg et al., 1986).

2.3.7 \textit{Yersinia enterocolitica}

\textit{Y. enterocolitica} infection were first recognised in Europe in 1963. Sporadic cases of human gastrointestinal infection due to
this organism have been reported with increasing frequency since 1966 (Highsmith et al., 1977). The infection is most commonly seen in infants and children (Mollaret et al., 1979). In parts of the Federal Republic of Germany and Canada, Y. enterocolitica rivals Salmonella and surpasses Shigella as a cause of acute enteritis (Marks et al., 1980).

In several tropical countries such as Iran, Israel, South Africa and Brazil, Y. enterocolitica has been observed to be a significant cause of human infections (Mollaret et al., 1979; Pizsolitto et al., 1979; Haghighi, 1979).

There are very few reports available on the isolation of Y. enterocolitica in the Indian sub-continent. Pramanik et al. (1980) first time reported isolation of Y. enterocolitica in India. Jain et al. (1983) from Indore reported its single isolation from a 1.5 year old female child suffering from loose motions with blood and mucus. In the same year, Singh et al. (1983) in a study of 157 infants and children with acute diarrhoea from Delhi found only 1 infant harbouring Y. enterocolitica serotype 0:4 from 1470 diarrhoeal faecal samples. There is a case report of serogroup 0:3 from Malaysia (Jegathesan et al., 1984). Recently a case of Y. enterocolitica infection in a child has been reported from Bangladesh (Neogi et al., 1985).

In Europe, Canada, Latin America, South Africa and Japan, serogroup 0:3 is most common (Bottone, 1977). For some unknown reasons; 0:3 and 0:9 strains are rare in USA while serogroup 0:8 predominates. Serogroup 0:9 strains are frequently found in some European countries (Swaminathan et al., 1982).
Y. enterocolitica produces a heat stable enterotoxin that like E. coli ST causes diarrhoea by activation of guanyl cyclase (Rao et al., 1981). The 17 C-terminal amino acid of Yersinia ST show 59% homology with both ST Ia and ST IIB of E. coli (Takao et al., 1984). The Yersinia ST gene is chromosomal in at least one strain of Y. enterocolitica (Robins et al., 1985).

2.3.8 Miscellaneous bacteria

Klebsiella is an ubiquitous organism which is isolated in a large proportion of both diarrhoeal and non-diarrhoeal stools. It is difficult, therefore to define its etiological role in diarrhoeal diseases.

K. pneumoniae have been associated with acute diarrhoea in children (Olarte et al., 1961; Weil et al., 1966). Bacteriological examination of clinical specimens from patients of acute and chronic diarrhoea, malnourished children and tropical sprue, revealed K. pneumoniae as the causative agent (Coello-Romirez et al., 1972; Gracey and Stone, 1973; Heyworth and Brown, 1975; Gorbach et al., 1975 and Kliipstein and Norowitz, 1975). K. pneumoniae isolates have been reported to produce enterotoxin as demonstrated in rabbit ileal loop (Kliipstein and Engert, 1976). It has recently been reported by Asnani and Jhanjee (1982) that K. pneumoniae enterotoxin not only induced a net flux of fluid and imbalance of electrolytes but also damaged the intestinal structure.

Klebsiella and Enterobacter have been reported to produce LT (Kliipstein and Engert, 1977). Antiserum to the Enterobacter LT was reported to partially neutralize the activity of Enterobacter.
E. coli and *Klebsiella* LTs but not CT. Antiserum to CT neutralized the activities of *E. coli* and *Klebsiella* LTs (Klipstein and Engert, 1977).

*E. cloacae, K. pneumoniae* and *S. flexneri* are known to produce STs that are active in the infant mouse assay (Klipstein and Engert, 1976; Ketyi et al., 1978). Two of the 47 *Klebsiella* strains isolated from human diarrhoea hybridized to the ST1b gene probe (Timmis et al., 1984).

*P. aeruginosa* is an opportunist as its infections are often hospital borne. Once this organism gains entry into the body, it is difficult to eradicate it owing to its resistance to most of the antimicrobial agents. *P. aeruginosa* has been implicated with food poisoning and was isolated from stools of diarrhoeal patients (Hunter and Eusign, 1967). Wadstrom (1978) reported that production of enterotoxin was common in stool isolates from Ethiopian children but a rare phenomenon in Swedish children with acute infantile diarrhoea. Similarly, Baljer and Barrett (1979) demonstrated that all 7 strains isolated from human food stuff were producing enterotoxin. Recently, food poisoning caused due to the consumption of stew contaminated with *P. aeruginosa* has been reported by Rokoszewska et al. (1980).

*P. aeruginosa* produces a heat labile enterotoxin (Kubota and Liu, 1971). This enterotoxin may be the same as the vascular permeability factor because each of 28 strains gave positive responses in the ligated loop and skin tests (Shriniwas et al., 1979). *Pseudomonas* may produce a heat stable toxin in addition to the heat labile enterotoxin. Out of 21 strains isolated from cases of
human diarrhoea, five hybridized to the E. coli STIb gene probe (Timmis et al., 1984).

Classical \textit{V. cholerae} has been known as a cause of both endemic and epidemic diarrhoea to mankind since antiquity. The eastern parts of India and adjoining Bangladesh are important endemic areas for cholera infection. Outbreaks of mild cholera due to \textit{Vibrio} elTor appear as the most important landmarks in the history (Chatterjee, 1979). Outbreaks of cholera infection have been reported from almost all parts of India, the most recent being at Indore which resulted due to contamination of drinking water by leakage in the sewage system (Prakash et al., 1981).

Thawani et al. (1982) analysed the faecal samples from cases of acute diarrhoea at different time interval and observed that maximum isolation rate of \textit{Vibrio} was within the first 12 hr of onset of disease.

During 1960 and 1970, \textit{V. cholerae} elTor was identified as a dominant strain causing endemic disease in Bangladesh. However since 1982, the classical strains of \textit{V. cholerae} are staging a comeback and at present both elTor and classical strains seem to be equally endemic in these areas (Huq et al., 1985).

Cholera has always been the bane of festival situations, where large number of people assemble. It was a special problem in Bangladesh refugee camps in 1970-71 (Mittal, 1986).

Non-cholera vibrios (NCV) or non-agglutinable (NAG) vibrios have the biochemical and fermentation properties similar to those of \textit{V. cholerae} but do not agglutinate with its polyvalent 'O' antiserum. There are 50 'O' serotypes (WHO, 1976). The widely
held technological view changed from another direction with ascribed role of NAG vibrios during "Kumbha" fair in India (Chatterjee, 1979).

Northern India is not generally considered endemic for cholera infection. However, sporadic cases of cholera are seen every year from June to September. Mittal et al. (1986) have reported isolation of both classical and NAG-vibrio.

A number of review articles in the recent years have dealt in depth about cholera toxin (Holmgren, 1981, Middlebrook and Dorland, 1984; Gemmell, 1984). Genetic and DNA hybridization studies of *V. cholerae* suggested that the genes for cholera toxin (CT) are not plasmid mediated like those of *E. coli* LT (Kaper and Levine, 1981). LT and CT cross-react antigenically and have similar structural and biochemical properties (Dallas and Falkow, 1979). DNA probes derived from cloned LT genes hybridize to toxinogenic but not to nontoxinogenic *V. cholerae* genomic DNA (Moselay and Falkow, 1980).

*V. parahaemolyticus* is now reported as a diarrhoeal agent from many countries of the world, especially in South East Asia (Sakazeki, 1971). Chatterjee and Neogy (1971) stated that owing to some special attributes viz, oxidase activity, negative ONPG test, gelation liquifaction, lysine decarboxylase reaction, motility at 37°C, marine ecology and halophilic nature, *V. parahaemolyticus* appears to occupy an intermediate taxonomic position between *Pasteurella* and *Yersinia*. 
A number of outbreaks of diarrhoea have been attributed to it in the USA (GDC, 1973). It is a frequent cause of gastroenteritis in Japan (Feeley and Balows, 1974). In a cholera endemic area where seafood is normally cooked before consumption, 11-15% cases admitted for treatment of diarrhoea were found to be due to this organism (WHO, 1976). In an area where *V. parahaemolyticus* infection is common, the organisms have been isolated from the hands of men selling fish and the flies caught in the market (WHO, 1976). Serotyping is the only reliable method for its epidemiological investigations. There are 11 somatic 'O' groups and 57 Capsular K antigens. All serotypes have an identical flagellar 'H' antigen, which is also shared by *V. algimolyticus* strains.

2.3.9 Non-bacterial diarrhoea

Rotavirus is a major etiological agent of non-bacterial acute diarrhoea in infants and young children (Bishop et al., 1973). Its infection in adults is usually milder to asymptomatic (Cubitt and Holzel, 1980). Human rotavirus (HRV) has an incubation period of 1 to 3 days. The diarrhoeal stools are usually watery without blood or mucus. Vomiting is a common feature and fever is usually of low grade. Rotavirus may precipitate a prolonged illness in infants because of its ability to cause lactose malabsorption (Davidson et al., 1984).

In India, only a few studies on rotavirus diarrhoea have been reported from different parts of the country. Holmes et al. (1974) detected rotavirus in 3 of the 5 children with gastroenteritis.
in Vellore. All the 10 specimens collected during an epidemic of childhood diarrhoea in Calicut, contained rotavirus particles (Paniker et al., 1977). Another study at Vellore, (Maiya et al., 1977), showed that 26% of hospitalized diarrhoea cases were associated with rotavirus. Yet another hospital based study in Calicut, (Paniker et al., 1982), showed a high prevalence of rotavirus diarrhoea in infants aged between 6 to 23 months.

Samantaray et al. (1982) examined diarrhoeal pre-school children from a semi-urban community and 99 hospital cases in Delhi and detected rotavirus in 21.2% and 32.3% cases respectively.

In Calcutta, rotavirus was detected in 5.6% diarrhoeal patients in the community (Sircar et al., 1984) and 22.4% in the hospitalized children (Saha et al., 1984).

Recently, Pupi and Rao (1986) studied neonatal rotavirus infection in Pondicherry using ELISA, and observed that 19% neonates with diarrhoea and 25% controls were positive. Female babies were infected more frequently than male (F:M = 2.4:1). The babies acquired the virus as early as on the third day after birth and the infection was more in under weight babies (2.5 kg) as compared to normal.

Rotavirus has two major antigenic specificities, i.e., subgroup and serotype. The former was demonstrated initially by an immune adherence haemagglutination assay (Kapikian et al., 1981) and the later by an ELISA and neutralization assay (Greenberg et al., 1983; Hashino et al., 1984; Taniguchi et al., 1984). Human rotavirus belongs to four serotypes and two subgroups namely I and II (Davidson, 1986).
Intestinal parasitic infection/infestations are widely prevalent in the developing countries. It has been estimated that 10% of the world population has either giardiasis or amoebiasis (Editorial, 1983).

*Giardia lamblia* is one of the common gastrointestinal parasites. Its prevalence varies from 1% to 30% (Petersen, 1972). In India, the prevalence rate is somewhat higher than in the West. A study in a rural community near Delhi showed that 12% of asymptomatic children and 23% of those with diarrhoea had *Giardia* infection (Ghai et al., 1969). Other studies gave a prevalence rate varying from 6.4 to 55 per cent (Tandon et al., 1972; Nair et al., 1977).

Most subjects with *Giardia* infection remain symptom free. Only a small percentage develops either an acute illness with severe diarrhoea and abdominal cramps (Wright et al., 1977) or a chronic diarrhoea with malabsorption (Chawla et al., 1975; Wolfe, 1977).

Harbouring of *E. histolytica* with or without clinical manifestation is termed amoebiasis (WHO, 1969). It lives as a commensal in the gastrointestinal tract without invading the tissue. Sometimes it can invade the bowel wall without producing any symptoms or even liver and other organs in the body.

Amoebiasis is less common in below 2 years of age, little more during thirties and maximum during forties and fifties (Shah, 1976). Male to female ratio varies among different countries being more common in males. Lower incidence in women,
particularly in Eastern countries, is believed due to relative abstinence from alcohol. Cases of amoebiasis are usually found throughout the year but more common during the summer months when the population of house flies increases.

Sargeant et al. (1984) reported that invasive isolates of *E. histolytica* can be differentiated by their electrophoretic isoenzyme pattern and now 22 zymodemes have been recognized.

*Cryptosporidium*, a protozoan parasite has recently been shown to be associated with diarrhoea in a number of mammalian species. Several cases of cryptosporidiosis in humans have been reported since 1976 (Nime et al., 1976; Heisel et al., 1976). The organisms lack host specificity and therefore is a potential zoonosis (Tzipori et al., 1981; Anderson et al., 1982). The majority of persons being immunologically compromised patients (Sloper et al., 1982; Tzipori, 1983). *Cryptosporidium* infection can be diagnosed by detection of oocyst in stool (Baxby and Blundell, 1983).