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Review of literature

All over the world, people wage a continual battle against food contamination and the resulting foodborne diseases. The history of attempts in alleviating the devastating consequences of food contamination is lost in the mist of past. Besides cooking, smoking and simple sun drying, fermentation is one of the oldest technologies used for food preservation. Over the thousands of years, it has evolved and has been refined and diversified. Today, a large variety of foods, produced on an enormous scale, are derived from this technology, which is used in households, small-scale food industries and large-scale entreprises (Table 1). Since the days of Louis Pasteur, who pointed to the importance of hygiene in relation to fermentation, it is established that this technology is easily influenced by various factors during processing, and if not followed correctly, the safety and/or quality of the final product may be jeopardized, leading to even outbreaks of foodborne illnesses.

In the tropical developing regions, like India, fermentation is one of the main options for processing foods. It serves as an affordable and manageable technique for food preservation. Fermentation can also increase the safety of foods by removing their natural toxic components, or by preventing the growth of disease-causing microbes (Nout 2001).

Table 1. Group of fermented foods

Group	Examples
Cereal	Ang-kak, banku, breads, gari, kenkey, lao-chao, mawè, ogi, beer
Legume	Dawadawa, inyu, kinema, meitauza, natto, papad, sufu, tempe, wadi
Cereal-legume mixture	Dhokla, idli, miso, soy sauces, taoco
Dairy	Cheeses, dahi, kefir, koumiss, lassi, yogurt
Fish	Bagoong, izushi, katsuobushi, som-fak
Meat	Country-cured ham, nem, nham, salami, pepperoni
Fruits and vegetables	Gundruk, kanji, kimchi, pickles, mesu, sauerkraut, sinki, wines
Miscellaneous	Basi, bongkrek, kishk, kombucha, miang, tarhana, ugba, vinegar

The antimicrobial factors present in fermented foods may affect both the growth and the survival of bacterial pathogens that are present in raw materials. In most fermented foods, the inhibition of growth is more common and can often ensure safety where levels of contamination are low. But with infectious pathogens, particularly those with a small minimum infectious dose, some degree of inactivation may be necessary to provide an acceptable level of safety. So, although fermented foods are generally considered as safe, process failures and contaminated raw materials have resulted in their being involved in foodborne illness. Several outbreaks of foodborne illnesses have raised questions regarding the safety of fermented products.

2.1. Legume-based fermented foods

The Indian population depends heavily on several fermented cereal products; many products are also made from combinations of cereal grains and legume seeds. This gives a higher protein content, and provides a better balanced ratio of amino acids, overcoming the danger of lysine deficiency from cereals alone or sulphur-containing amino acid deficiency from legumes alone. These cereal-legume products include idli, dosa, dhokla, adai, vada, papadam and wadi (Padmaja and George 1999). The popularity of fermented foods is due to the desirable changes in texture and taste brought about during fermentation, not to mention the improvement in digestibility (Ramakrishnan 1979b). Although extensive study on the microbiology and biochemistry on fermentation of some of these foods have been done, no due attention has been paid to the microbiological quality in respect to the foodborne bacterial pathogens that might be present in those foods. Relevant information on microbiology and biochemistry of these foods found in the available literatures is summarized below.

2.1.1. Dhokla

Dhokla, one of the popular indigenous fermented foods of India, had its origin in Gujarat. It is liked as snacks all over the country because of its unique mild sour taste and a spongy texture. Traditionally, it is prepared by fermenting a mixture of Bengalgram (*Cicer arietinum*) dal and rice (*Oryza sativa*) (Nout *et al.* 2007).

The microbiota associated with the fermentation of dhokla batter consists of lactic acid bacteria and yeasts. The different types of lactic acid bacteria identified were *Lactobacillus fermentum, Lactococcus lactis, Lactobacillus delbrueckii* and *Leuconostoc mesenteroides*. However, *L. lactis and L. delbrueckii* become extinct after 12 and 15 h, respectively, of fermentation. The yeast identified was *Pichia silvicola*. The pH of dhokla batter falls from 5.3 at the start to 4.0 after 18 h of fermentation. Total titratable acidity increases indicating production of acids by the proliferating micro-organisms. Diacetyl and

acetoin content increase, but during prolonged fermentation diacetyl may be converted to 2-3-butanediol. The flavour of the product could be attributed to acetoin and volatile fatty acids, namely acetic acid, propionic acid, iso-butyric acid and iso-valeric acid. The lactic acid bacteria contribute lactic acid and acetoin. The yeast produces folic acid and raises the volume of the batter, imparting sponginess to the product (Joshi *et al.* 1989; Kanekar and Joshi 1993).

2.1.2. Dosa

Dosa is a fried, thin, fairly crisp, pancake-like food prepared from a mixture of milled rice and blackgram (*Phaseolous mungo*) dal having its origin in South India. Ordinarily the micro-organisms developed during the initial soak is sufficient to bring about the fermentation (Soni *et al.* 1985, 1986). Dosa batter is a slightly modified version of idli batter, where the proportion of rice is increased and both the ingredients are finely ground and the batter is made to a thinner consistency than the idli batter. Sometimes the batter is added with backslop (fermented batter of the previous batch which serves as a starter). Dosa is prepared by spreading the fermented batter on a hot greasy griddle (Aidoo *et al.* 2006; Padmaja and George 1999). The microbiota of the fermenting batter consists of either bacteria alone or, less frequentiy, bacteria in combination with yeasts. The loads of bacteria and yeasts after 24 h fermentation were 10^9 and 10^6, respectively, g^{-1} dry matter (Soni *et al.* 1985). *L. mesenteroides* is the most commonly encountered bacterium followed by *Enterococcus faecalis* and *L. fermentum, Bacillus amyloliquefaciens, L. delbrueckii, Bacillus subtilis, Pediococcus cerevisiae, Bacillus polymyxa and Enterobacter* sp. Of the yeasts, *Saccharomyces cerevisiae* is most predominant followed by *Debaryomyces hansenii, Pichia anomala, Trichosporon cutaneum, Oosporidium margaritiferum, Guehomyces pullulans, Kluyveromyces marxianus, Candida kefyr and C. krusei* (Padmaja and George 1999). However, yeasts are absent when the ingredients are ground without presoaking (Soni *et al.* 1986). The pH of dosa batter declines from 5.5 to 4.1 after 24 h of fermentation during which there is an increase in batter volume and contents of soluble solids, reducing sugars, non-protein nitrogen, soluble nitrogen, free amino acids, thiamine, riboflavin and cyanocobalamine (Padmaja and George 1999). Soluble protein and reducing sugar contents, though decline initially, increase significantly after 24 h of fermentation (Soni *et al.* 1985).

2.1.3. Idli

Though originated in the southern part of India, idli has gained rapid popularity all over the country (Ramakrishnan 1979a). It is a moist steamed tiffin food, prepared by fermenting a mixture of wet milled rice (raw or parboiled) and blackgram dal. During preparation, the ingredients are washed and soaked separately in water. The soaked rice is then coarsely ground, whereas the dal is ground finely to a smooth mucilaginous paste. Then two slurries (2:1) are mixed with salt, put in a closed container and left overnight to allow a definite leavening (a two- to three-fold increase in the original volume) and to develop a pleasant acid flavour. The fermented batter is poured in cups of an idli pan and steamed until the idli cakes are soft and spongy with a honeycomb structure inside (Aidoo *et al.* 2006; Nout *et al.* 2007; Steinkraus *et al.* 1967). The honeycomb structure develops due to escape of gases trapped by the abundant gluten present in blackgram. Variation is there in the ratio of the two ingredients depending on availability of raw materials and consumer preference. Proportions of rice to blackgram varies from 4:1 to 1:4 in various studies (Desikachar *et al.* 1960; Lewis and Johar 1953; Mukherjee *et al.* 1965). Higher amounts of rice imparts a starchy flavour to idli. Soaking period varies from 3-10 h. Lewis reported that the best quality idli was prepared when ingredients were soaked and ground with water at 80 °C (Lewis and Johar 1953). But it takes a longer fermentation period due apparenfly

to destruction of some of the micro-organisms initially present (Steinkraus *et al.* 1967). Fermentation period varies from 14 to 24 h, overnight being the best frequent time span for idli. Substitutes of blackgram include dehulled dark soya beans (*Glycine max*) and common beans (*Phaseolus vulgaris*). It produces satisfactorily idli with higher protein content and idli-like product that might be useful in international food studies (Steinkraus *et al.* 1967). A strain of *L. mesenteroides* was isolated from soya bean idli which secrets a α-N-acetyl-glucosaminidase and α-D-mannosidase which hydrolyze the haemagglutinin in soya bean (Ramakrishnan 1979b).

Idli is a natural fermented food, no inoculum is added. The nutritional value of idli depends on the micro-organisms inherent in the food material used, raw materials and conditions of fermentation (Ramakrishnan 1979b). The composition of microbiota involved in idli batter fermentation is a controversial point. The micro-organisms present on the blackgram were sufficient to produce the required leavening and acid within a reasonable time interval. Whereas no fermentation failures were encountered with added inocula of yeast and/or sour buttermilk, occasional failures were encountered without the inocula. Mukherjee *et al.* (1965) reported sequential changes of bacterial flora. The predominant micro-organisms responsible for souring as well as gas production was *L. mesenteroides* followed by *E. faecalis* and *P. cerevisiae* in succession. Steinkraus *et al.* (1967) reported an initial bacterial load of the batter of 10^3-10^5 cells ml^{-1}, which after 20-22 h of fermentation increased up to 10^8-10^9 ml^{-1}. Predominant micro-organisms in idli batter are *L. mesenteroides* and *E. faecalis*. *Pediococcus* is also infrequently encountered. Other bacteria involved include *L. fermentum* and *L. delbrueckii*. Besides lactic acid bacteria, yeasts, such as *Candida saitoana, G. pullulans and T. holmii* were isolated from ripe batter and apparently they play some role in idli fermentation (Batra and Millner 1974; Lewis and Johar 1953; Venkatasubbaiah *et al.* 1984). Venkatasubbaiah *et al.* (1985) have reported four genera of yeasts including *Hansenula, Candida, Torulopsis* and *Trichosporon* from batter samples procured from hotels. Soni and Sandhu (1991) are of opinion that *S. cerevisiae* enrichment in combination with natural bacterial flora of the ingredients is the best microbial factor for standardizing idli fermentation in terms of improved organoleptic characteristics, leavening and nutritional constituents During fermentation, predominant among the yeasts appearing first are *S. cerevisiae, D. hansenii, P. anomala* and *G. pullulans*; and *T. cutaneum* develops subsequently (Aidoo *et al.* 2006). Fermentation of the batter during preparation of idli is responsible for increase in total acids and soluble solids but decrease of soluble nitrogen and total nitrogen (Steinkraus *et al.* 1967). The initial pH of 6.0 drops to 4.3-5.3. Though pH of 4.3-4.7 seemed desirable, idli prepared from batter with pH 4.1-5.3 had a satisfactory flavour when steamed. Venkatasubbaiah *et al.* (1984) found that yeast-fermented batter produced carbon dioxide, whereas batter fermented with *L. mesenteroides* produced hydrogen only.

2.1.4. Papad

Papad, popularly called papadam in southern parts of India, is a condiment prepared from blackgram dal. This thin, usually circular, wafer-like product is used to prepare curry or eaten by itself as a crackly snack or appetizer with meals after roasting or deep-frying in oil. Papad-making under controlled conditions has already developed into a cottage or small-scale industry (Aidoo *et al.* 2006). A variety of papads are produced from a great diversity of ingredients. They are made either using only cereal flour or a combination of it and pulse flour. Blackgram flour, lentil (*Lens culinaris*), redgram or greengram (*Vigna radiata*) flour is hand-kneaded with a small quantity of peanut oil, common salt (~ 8 g kg^{-1}), 'papad khar' (saltworts produced by burning a variety of plant species, or from very alkaline deposits in the soil) and water, and then pounded to a stiff paste. The dough (sometimes with a backslop and spices added) is left to ferment for 1-6 h. The fermented dough is shaped into small

balls which are rolled into thin, circular flat sheets (10-24 cm diameter, 0.2-1.2 mm thick) and generally dried in the shade to 12-17% (w w^{-1}) moisture content. (Aidoo *et al.* 2006; Saxena *et al.* 1989; Shurpalekar and Venkatesh 1975). The diameter and thickness of standard papad should be 5-23 cm and 0.1-1.2 mm, respectively (ISI 1972).

Candida krusei and *S. cerevisiae* are involved in the preparation of papad (Shurpalekar 1986). Moisture content of raw commercial papads ranges from 9.0 to 17.7%, total ash from 7.2 to 11.8%, acid insoluble ash from 0.18 to 0.52%, ether extractives from 2.2 to 5.8%, alkalinity from 3.4 to 6.0%, pH from 7.4 to 9.1, protein from 9.8 to 19.2%, free fatty acid content (as oleic acid) from 0.51 to 0.58% with diametric expansion on deep fat frying of papads from 4.8 to 17.8% (Kulkarni *et al.* 1996).

2.1.5. Wadi

Wadis, traditionally consumed in Punjab and Bengal of India, are now popular in many places of India, Pakistan and Bangladesh. Wadis are dried, hollow, brittle cones or balls (3-8 cm in diameter, 15-40 g in weight). It is used as a spicy condiment or adjunct for cooking vegetables, grain legumes or rice (Aidoo *et al.* 2006). To prepare wadi, generally blackgram dal is soaked, drained, ground into a smooth soft dough, left to ferment for 1-3 days and moulded into cones or balls which are deposited on bamboo or palm mats smeared with oil and sun-dried for 4-8 days. The surface of the cones or balls becomes covered with a mucilaginous coating which helps to retain the gas formed during their fermentation. The wadis look hollow, with many air pockets and yeast spherules in the interior and a characteristic surface crust. Sometimes spices like asafoetida, caraway, cardamom, cloves, fenugreek, ginger and red pepper are added to the fermenting dough (Aidoo *et al.* 2006; Sandhu and Soni 1989). Several bacteria and yeasts, constituting the natural flora of blackgram, spices and surroundings, are associated with wadi fermentation. The bacteria appearing initially in traditional wadi fermentation are *L. mesenteroides, L. delbrueckii, L. fermentum* and *B. subtilis* along with *Flavobacter* spp. *E. faecalis* develops subsequently. However, only *L. mesenteroides* and *L. fermentum* prevail at the end of fermentation. Among yeasts, the predominant ones are *T. cutaneum, S. cerevisiae, C. krusei, Pichia membranefaciens* and *P. anomola,* but eventually only *S. cerevisiae* and *T. cutaneum* persist. The development and prevalence of microbiota are affected by the seasons, summer being more favourable for bacteria and winter for yeasts. The lactic acid bacteria are mainly responsible for the acidification of dough, favourable conditions for the yeasts to grow and become active for leavening (Aidoo *et al.* 2006). During fermentation, the pH declines from 5.6 to 3.2 with the accompanying increase in total acid levels, dough volume and contents of soluble solids, non-protein nitrogen, soluble nitrogen, free amino acids, proteolytic activity and B-vitamins (Batra and Millner 1974; Sandhu and Soni 1989; Sandhu *et al.* 1986).

2.2. Foodborne bacterial pathogens

2.2.1. *Bacillus cereus*

B. cereus was originally isolated and described by Frankland and Frankland (1887). Anecdotal evidence of *B. cereus* food poisoning had existed in Europe since the turn of the last century. One of the earliest recorded episodes of *B. cereus* food poisoning was that of 1906 when Lubenau (1906) described an outbreak in a sanatorium in which 300 of 400 inmates and staff became ill with profuse diarrhoea, stomach cramps, and vomiting shortly after eating meatballs in the dinner. A large number of aerobic sporeforming *Bacillus* originally reported by him as *Bacillus peptonificans,* although most probably

should be identified as a strain of *B. cereus*, was isolated from the remnants of the dish. Later Seitz (1913), Brekenfeld (1926, 1929) and Trub and Wundram (1942) reported that *Bacillus*-contaminated foodstuffs stored for long periods at improper temperatures were able to cause illness when eaten. During the period 1936-1943, 117 of 367 cases investigated by the Stockholm Board of Health were suspected of being caused by aerobic sporeformers (Plazikowski 1947). Confusion was there in the early nomenclatures during reporting of *Bacillus*-related food poisoning. This reflected the disorder in *Bacillus* taxonomy as well as contributed to the slow recognition of pathogenicity among members of the genus *Bacillus* other than *B. anthracis*. Full credit goes to Smith *et al.* (1952) and Gordon *et al.* (1973) in bringing order to *Bacillus* taxonomy, and in providing a foundation of tests and interpretations on which rests our present understanding of this group of micro-organisms. However it was not until Hauge's remarkable experiments in the 1950s (Hauge 1950, 1955) that *B. cereus* was established as a cause of food poisoning. He presented the first classic description of *B. cereus* gastroenteritis in his discussion of four Norwegian outbreaks involving 600 persons resulting from vanilla sauce prepared and stored at room temperature for one day before being served. Later Hauge consumed vanilla sauce containing 10^7-10^8 cells ml^{-1}, and within 16 h he was suffering from profuse diarrhoea accompanied by cramping abdominal pain.

Cells of *B. cereus* are large (cell width, > 0.9 μm), gram-positive rods, and motile by means of peritrichous flagella. Single central to terminal, ellipsoid or cylindrical endospore without distention of the sporangium is present. *B. cereus* is able to metabolize glucose, fructose and trehalose, but not pentoses and many of the sugar alcohols. A small percentage of strains are urease-positive; the majority actively hydrolyzes starch, casein, and gelatin (Gordon *et al.* 1973; Granum 2007).

Growth and multiplication of vegetative *B. cereus* cells typically occur within 10-50°C, with the optimum between 28° and 30°C. However, psychrophilic variants identified in raw milk samples can grow at temperatures as low as 5 °C. An initial-stage spore germination has been demonstrated within wider limits, i.e., -1 °C, 30 °C and 59 °C being the minimum, optimum and maximum temperatures, respectively (Knaysi 1964). The range of pH permitting growth of *B. cereus* in laboratory media has been reported to be 4.9 to 9.3 when adjusted with mineral acids and alkalis (Fluer and Ezepchuk 1970; Kim and Goepfert 1971). Minimum water activity (a_w) for growth of *B. cereus* was reported to be 0.95, however in Cantonese style fried rice, the a_w ranged from 0.912 to 0.961 (Kramer and Gilbert 1989).

One of the most important aspects of study is the heat resistance of *B. cereus* spores because spore is a factor of primary concern to the food and pharmaceutical industries (Kramer and Gilbert 1989). Since spore inactivation is the principal concern in producing appertized foods, much higher temperatures are used in appertization processes and in the measurement of spore *D*-value (decimal reduction time). The *D*-value is defined as the time at a given temperature for the surviving population to be reduced by 1 log cycle, and *z*-value is defined as the temperature change which results in a 10-fold (1 log) change in *D* (Adams and Moss 1995).

D-values at 85 °C, 90 °C, 95 °C and 100 °C in phosphate buffer (pH 7.0) was reported to be 220 min, 71 min, 13 min and 8 min, respectively (Mol 1957). In contrast, $D_{121°C}$-values in soya bean oil and olive oil were 30 and 17.5 min, respectively, demonstrating that lipid material have a protective effect on the thermal resistance of *B. cereus* spores (Molin and Snygg 1967). In low acid foods (> pH 4.5), the $D_{100°C}$-value was found to be 5 min (Ingram 1969). A $D_{100°C}$-value of 2.7-3.1 min for *B. cereus* in skim milk was reported by Mikolajcik (1970). *z*-values ranging from 6.7 to 8.3°C were obtained in aqueous spore suspension by Gilbert *et al.* (1974). Strains (serotype H.1) producing emetic syndrome food poisoning are more heat resistant ($D_{95°C}$: 22.4-36.2 min) compared to routine isolates of *B. cereus* (random serotype) from samples of raw rice ($D_{95°C}$: 1.5-6.0 min) (Parry and Gilbert 1980). $D_{100°C}$ and $D_{92°C}$-values for six isolates in rice broth were 4.2-6.5 min and 16-36 min, respectively (Chung and Sun 1986). Rajkowski

and Mikolajcik (1987) reported $D_{100°C}$-values of *B. cereus* ranging from 0.6 to 27.0 min in demineralized water. The heat resistance at 90 °C for spores of the 32 *B. cereus* strains isolated from fresh vegetables and refrigerated minimally processed foods ranged from 1.4 to 21.2 min. $D_{90°C}$, $D_{95°C}$ and $D_{100°C}$ were 3.2-23.3, 0.7-5.2 and 0.4-1.1 min, respectively, in strains isolated from Spanish raw rice (Sarrias *et al.* 2002). Strains unable to hydrolyze starch were the most heat-resistant, with $D_{90°C}$-values higher than 10.8 min (Valero *et al.* 2002). Several studies have provided evidence of non-linear spore survivor curves associated with certain strains. Aging of spores affects the *D*-value (Collado *et al.* 2003). Banerjee and Sarkar (2004a) reported the $D_{100°C}$-values of *B. cereus* isolates from spices ranging from 3.5 to 5.9 min in glucose-supplemented brain-heart infusion broth.

Other factors that have been shown to exert a total inhibitory effect on the growth of *B. cereus* include 2 g sorbic acid kg^{-1} rice filling of Karelian pastry (Raevuori 1976), 500 µg benzoic acid ml^{-1} (pH 6.3) (Lueck 1980) and 6-10 mg garlic aqueous extract g^{-1} (Banerjee and Sarkar 2003). Application of nisin at the levels of 5 mg l^{-1} has been shown to act as an effective preservative giving significant increase in shelf-life and providing protection against the growth of psychrotrophic *B. cereus* (Delves-Broughton *et al.* 1992). Addition of nisin to a batter of crumpets at levels of 3.75-6.26 µg g^{-1} effectively prevented the growth to levels capable of causing food poisoning (Jenson *et al.* 1994).

The antibiotic susceptibility was tested for 66 isolates of *B. cereus* from rice in Taiwan against 12 different antibiotics. The isolates were 100% susceptible to chloramphenicol (30 µg $disc^{-1}$), erythromycin (15 µg $disc^{-1}$) and streptomycin (10 µg $disc^{-1}$), and 92.4% were sensitive to gentamicin (10 µg $disc^{-1}$). However, they were 100% resistant to penicillin G (10 units $disc^{-1}$) and polymyxin B (300 units $disc^{-1}$), 99% resistant to ampicillin (10 µg $disc^{-1}$) and carbenicillin (100 µg $disc^{-1}$) and 88% resistant to cephalothin (30 µg $disc^{-1}$) (Chung and Sun 1986). Shah *et al.* (1996) isolated *B. cereus* from about 300 samples of a variety of foods in which *B. cereus* was found in 20% spices. The antibiogram pattern of *B. cereus* was obtained with 50 isolates against nine antibiotics. All the isolates were resistant to ampicillin (10 µg $disc^{-1}$). A high resistance was found against trimethoprim (5 µg $disc^{-1}$) (92%), colistin (10 µg $disc^{-1}$) (86%) and rifampicin (5 µg $disc^{-1}$) (92%). All the isolate were sensitive to chloramphenicol (30 µg $disc^{-1}$) and ciprofloxacin (5 µg $disc^{-1}$), and 88% sensitivity was seen against streptomycin (10 µg $disc^{-1}$) and vancomycin (30 µg $disc^{-1}$).

B. cereus produces several phospholipases, eg. phospholipase C and egg yolk turbidity factor, with preferences for different phospholipids, including phosphatidylcholine (~ 23 kD), phosphatidylinositol (~ 29-35 kD), and a sphingomyelinase (~ 29 kD) (Drobniewski 1993).

B. cereus causes two different types of food poisoning: (i) the diarrhoeal type, first recognized after a hospital outbreak associated with vanilla sauce in Oslo, Norway, in 1948 (Hauge 1955), and (ii) the emetic type, described about 20 years later after several outbreaks associated with fried rice in London (Mortimer and McCann 1974). The emetic toxin, causing emesis (vomiting), is produced (preformed) by growing cells in the food (Kramer and Gilbert 1989), while the diarrhoeal type of food poisoning is caused by a complex enterotoxin produced during vegetative growth of *B. cereus* in the small intestine (Granum 1994). For both types of foodborne illness the food involved has usually been heat-treated, and surviving spores are the source of the food poisoning . The emetic toxin (1.2 kD) has been named cerulide, and consists of a ring structure of three repeats of four amino acids or oxy acids. Cerulide is resistant to heat (90 min at 121 °C), pH and proteolysis, but is not antigenic. Diarrhoeal disease is caused by at least three different enterotoxins, viz. haemolysin (Hbl), non-haemolytic enterotoxin (Nhe) and CytK. Two of the enterotoxins are multicomponent, one haemolytic and the other non-haemolytic, while the third (CytK) is a single protein (Granum 2007). The so-called 'diarrhoeal syndrome' is usually associated with proteinaceous foods, vegetables, sauces and puddings. The syndrome is characterized by an incubation period within the range of 8-16 h (av. 10-12 h) before the onset of abdominal pain, profuse watery diarrhoea, rectal tenesmus and occasional nausea that seldom

results in vomiting. Symptoms generally resolve within 12-24 h. In contrast, the 'emetic syndrome' form of illness is almost exclusively associated with farinaceous foods, particularly cooked rice, and is characterized by rapid onset (1-5 h) of nausea, vomiting and malise, in some cases followed by diarrhoea, of 6-24 h duration (Kramer and Gilbert 1989).

B. cereus is not a competitive micro-organism, but grows well after cooking and cooling (< 48 °C). The heat treatment causes spore germination, and in the absence of competing flora, *B. cereus* grows well, with a generation time as low as 12 min under optimal conditions (Borge *et al.* 2001). The infective dose of *B. cereus* sufficient to induce illness is variable. It was shown that count of *B. cereus* in foods ranging from 4.5-9.0 log cfu g^{-1} resulted in enteritis. Further, occurrence of *B. cereus* (> 10^4 cfu g^{-1}) in food has been considered as one of the epidemiological criteria for implicating *B. cereus* in food poisoning outbreaks (Concon 1988). Johnson (1984) reported a population of > 10^5 *B. cereus* cells g^{-1} is required for a food poisoning outbreak to occur.

Measures recommended for prevention of *B. cereus* gastroenteritis includes keeping foods at a temperature where the spores do not germinate and cells do not grow, proper sanitary measures during handling to prevent post-preparation contamination, and uniform reheating of a suspected food to above 75 °C before serving (Ray 2001).

2.2.2. *Clostridium perfringens*

The first description of *C. perfringens*, formerly known as *C. welchii*, was given by Welch and Nuttal (1892). However, it was not until 1940 that Knox and MacDonald in England confirmed *C. perfringens* as a cause of food poisoning. In addition to enteritis, *C. perfringens* is responsible for necrotizing tissue infections. Historically, the organism is best known for its association with gas gangrene (Adams and Moss 1995).

C. perfringens is a typical gram-positive, rod shaped (1 μm x 3-9 μm), anaerobic, sporeforming (oval in shape, subterminal in position) bacterium that is encapsulated and non-motile. Though catalase-negative, it survives and occasionally grows in the presence of oxygen (Labbe 1989). Growth of *C. perfringens* occurs at a temperature of 2-50 °C, although it is very slow below 20 °C. The most important characteristic of *C. perfringens* relative to food safety is the organism's ability to grow optimally at elevated temperatures ranging between 43 and 45 °C. However, Willardsen *et al.* (1978) found that one strain (NCTC 8238) has a shorter generation time of 7.1 min at 41 °C than at 45 °C. Minimum pH for growth is 5.0, whereas 6.0-7.5 is found to be optimum. Minimum a_w for growth is 0.95-0.97. Tompkin *et al.* (1974) found an inhibitory effect of sorbic acid on *C. perfringens*. Control of *C. perfringens* was achieved in Italian sausage by incorporating 5 μg nisin g^{-1} (Caserio *et al.* 1979). Growth of most strains of *C. perfringens* is prevented by sodium chloride at a level of 70-80 g kg^{-1}, although some inhibition occurs at a level of 50-60 g kg^{-1} (Roberts and Derrick 1978).

Food poisoning due to *C. perfringens* is usually self-limiting. Non-febrile illness characterized by nausea, abdominal pain, diarrhoea and, less commonly, vomiting usually occurs 8-24 h after the ingestion of food. The minimum required ingested dose of *C. perfringens* has been variously estimated at 10^6-10^8 cfu g^{-1}. However, the median count of the pathogen in foods implicated in outbreaks in UK was 7×10^5 g^{-1}. In otherwise healthy individuals, medical treatment is not usually required and recovery is complete within 1-2 d, although occasional fatalities occur in the very old or debilitated persons (Adams and Moss 1995; CDC 1985; Shandera *et al.* 1983).

C. perfringens is classified into five types, designated A through E, based on the production and expression of four (α, β, ϵ and ι) 'typing' toxins (McClane and Rood 2001). Type A strains are involved in foodborne intoxication. The enterotoxin associated with the foodborne disease is a heat-

labile protein. This intracellular protein is produced by the cells during sporulation in the intestine and released. There are some reports that, in addition to the intestine, sporulation and enterotoxin production to certain levels can also occur in some foods (Garvani 1987; Labbe 1988). The enterotoxin has been shown to be the major virulence factor in the common form of food poisoning. Stark and Duncan (1971) first showed that all clinically significant properties were linked to the enterotoxin. Human volunteer studies strengthened the theory (Skelkvåle and Uemura 1977), and gene deletion studies gave the definitive proof that the effects seen are solely due to the production of enterotoxin (Brynestad and Granum 2002).

C. perfringens is a common cause of food-poisoning outbreaks in USA (CDC 1985). There in the 1960s and 1970s, *C. perfringens* was involved in over 7% of the total foodborne outbreaks and over 10% of the total number of cases. In the year 1980s, the incidence dropped to about 3% of total outbreaks, affecting about 5% of the total cases. Most of the outbreaks generally occurred from cafeterias, restaurants, schools and banquets (Bean and Griffin 1990). Between 1980 and 1990, outbreaks in England and Wales have numbered between 46 and 69 each year with 896 to 1624 cases; corresponding figures for Scotland over the same period were 5 to 11 outbreaks and 75 to 364 figures (Adams and Moss 1995).

Growth of *C. perfringens* in food is influenced by a variety of environmental factors, including temperature, E_h, pH and a_w. The heat-resistance property of spores in part contributes to the ability of *C. perfringens* to cause food poisoning by enabling this bacterium to survive in undercooked foods. Spores of food poisoning isolates are typically much more heat-resistant than spores of *C. perfringens* isolates obtained from other sources; the extreme heat resistance of food poisoning isolate spores may contribute to foodborne virulence. It is also noteworthy that incomplete cooking of foods may not only fail to kill *C. perfringens* spores in foods but also actually favours development of *C. perfringens* type A food poisoning by inducing spore germination (McClane 2007). Roberts (1968) reported that spores of heat-resistant strains had $D_{90°C}$-values of 15-145 min and *z*-values of 9-16 °C, whereas spores of heat-sensitive strains had $D_{90°C}$-values of only 3-5 min and *z*-values of 6-8 °C. Furthermore, spores of heat-resistant strains required heat activation at 78-80 °C, whilst up to 50% of heat-sensitive strains grew without heat activation (Crowther and Baird-Parker 1984). *D*-values of spores of *C. perfringens* show a wide inter-strain variation with recorded values from 0.31 to > 38 min. The *D*-values of spores of NCTC 8798 at 95 °C, 105 °C and 120 °C were found to be 52.7 min, 2.5 min and < 0.01 min, respectively, for in water as the heating medium. At 90 °C and 100 °C, *D*-values for strain NCTC 10240 were found to be 15.5 min and 0.2 min, respectively, but for ATCC 3624, the values were 27.5 min and 0.22 min, respectively (Labbe 1989). Resistance of spores to heat is less in phosphate buffer than in water but greater in cooked meat than in water (Sutton 1966). Besides genetic difference among strains, lots of other factors including sporulating medium and heating medium, and contamination with vegetative cells and sporangia contribute to the variation in *D*-values (Labbe 1989). The antibiotic susceptibility of *C. perfringens* strains isolated from meat are similar to clinical isolates suggesting that meat is not a common source of resistant or multiple-resistant strains. However, porcine isolates from strains isolated from swine grown on farms where antibiotics are commonly used are often resistant to multiple antibiotics (Rood *et al.* 1978), one of which is transferable. Penicillin G has been the drug of first choice for treatment of gas gangrene by *C. perfringens*. Generally antibiotics are not used in case of human food poisoning. Recent reports of antibiotic-associated diarrhoea caused by *C. perfringens* are there.

The lowest a_w supporting growth of *C. perfringens* is 0.93 when other growth conditions are near-optimal. Although *C. perfringens* is an anaerobe, it does not require an extremely reduced environment to grow. Provided the environmental E_h is suitably low for initiating growth, *C. perfringens* can produce reducing molecules such as ferredoxin to modify the E_h of its environment and create

favourable growth conditions. In fact, the E_h of many common foods such as raw meats and gravies is often adequate to support the growth of *C. perfringens* which is pH sensitive, with optimal growth being at 6-7. It grows slowly, if at all, at pH values of < 5 and > 8.3 (Labbe 1989).

C. perfringens is widely distributed throughout the natural environment, including soil, foods, dust and the intestinal tract of humans and domestic animals (Labbe 1989). However, it is now understood that < 5% of global *C. perfringens* isolates carry the enterotoxin gene (*cpe*) necessary for causing *C. perfringens* type A food poisoning (McClane 2007).

In virtually all outbreaks the principal cause is failure to refrigerate properly previously cooked foods, specially when prepared in large portions. Rapid and uniform cooling of foods is therefore imperative. Gravies, broths and large pieces of meat should be cooled to < 10 °C within 2-3 h. Cooked, chilled foods should be related to minimal internal temperature of 75 °C immediately before serving to destroy vegetative cells. Cooked meat should be kept above 60 °C or below 10 °C. As most people harbour *C. perfringens* in their intestinal tract, preventing carriers from handling food is rather impossible. Similarly, the organism is present in a wide variety of foods. So, education of the food handlers remains a critical aspect of *C. perfringens*-related food poisoning control (Labbe 1989).

2.2.3. *Staphylococcus aureus*

The staphylococci were first described by Ogston (1881) as a pyogenic infection in humans. Staphylococcal food poisoning is among the most prevalent causes of gastroenteritis worldwide. It results from ingestion of one or more preformed staphylococcal enterotoxins in *Staphylococcus*-contaminated food. The etymological agents are members of the genus *Staphylococcus*, predominantly *S. aureus*. This form of food poisoning is called intoxication, and does not involve infection by, and growth of, the bacteria in the host. Staphylococcal toxin, the first true enterotoxin to be described, is not entirely destroyed by heating even for 30 min at 100 °C (Seo and Bohach 2007).

S. aureus cells are small (0.5-1.5 μm in diameter), gram-positive spheres. Cell division in more than one plane results in irregular clumps resembling bunches of grapes. They are catalase-positive, oxidase-negative, facultative anaerobic, and can ferment glucose. Most strains ferment mannitol and produce coagulase, thermonuclease, and hemolysin. The cells are killed at 66 °C in 12 min, and at 72 °C in 15 s. The suitable temperature for growth ranges from 7 to 48 °C, with fairly rapid growth in between 20 and 37 °C. Growth occurs optimally at pH 6.0-7.0, with minimum and maximum limits of 4.0 and 9.8-10.0, respectively. It grows rapidly in media containing sodium chloride at a level of 50-70 g kg^{-1}, and some strains are capable of growth in up to 200 g 100 kg^{-1} (Adams and Moss 1995). Lahellec *et al.* (1981) reported that the growth of *S. aureus* in brain heart infusion (pH 5.0) was inhibited by 10 g sorbate l^{-1}, but at pH 7.0 the organism grew in the presence of 50 g sorbate l^{-1}. Tompkin *et al.* (1974) reported inhibition of *S. aureus* by sorbic acid in cooked uncured sausage. The bacterium is sensitive to nisin (Thomas *et al.* 2000). It grows down to a_w 0.83 where it has a generation time of 300 min (Adams and Moss 1995).

S. aureus is wide spread, but occurs most frequently on the skin of higher primates. In humans, it is particularly associated with the nasal tract where it is found in 20-50% of healthy individuals. It can be isolated from faeces and sporadically from a wide range of other environmental sites such as soil, marine and fresh water, plant surfaces, dust and air (Adams and Moss 1995).

In USA, between 1983 and 1987, staphylococci accounted for 7.8% (47) of the 600 bacterial food poisoning outbreaks that were recorded. Equivalent figure for England and Wales over the same period was 1.9% (54) out of a total of 2815 outbreaks. Outbreaks of staphylococcal food poisoning inthe UK peaked during the 1950s at 150 outbreaks per year but have since declined to an annual levelof 10-20 outbreaks (Adams and Moss 1995).

Food poisoning by *S. aureus* is characterized by a short incubation period, typically 2-4 h. Nausea, vomiting, stomach cramps, retching and prostration are the predominant symptoms, although diarrhoea is also often reported and recovery is normally complete within 1-2 d. In severe cases, a marked dehydration may require treatment by intravenous infusion (Adams and Moss 1995).

The short incubation period is characteristic of toxins preformed in foods. *S. aureus* produces seven protein exotoxins designated A, B, C_1, C_2, C_3, D and E. The molecular mass of them are 27.1, 28.366, 27.496, 27.531, 27.438, 26.360 and 26.425 kD, respectively. The toxins are single-chain polypeptides each containing a single disulfide loop near the molecule's centre. As a result of their compact structure they are resistant to gut proteases and heat stable, being inactivated only by prolonged boiling. Toxin types A and D, either singly or in combination, are most frequently implicated in outbreaks of food poisoning. In the UK, type A is responsible for 52% of outbreaks, type D for 6%, types A and D combined for 19%, and types C and D combined for 9%. Susceptibility varies between individuals but it has been estimated that in outbreaks <1 µg of pure toxin has been required to elicit symptoms (Adams and Moss 1995).

Except for clinical isolates such as some community-acquired methicillin-resistant *S. aureus* (MRSA) strains exposed to antimicrobial therapy, most staphylococci are sensitive to β–lactams, tetracyclines, macrolides, lincosamides, novobiocin and chloramphenicol but are resistant to polymyxin and polyene (Stefani and Varaldo 2003).

Humans are the main reservoir for staphylococci involved in human disease. In humans, the anterior nares are the predominant site of colonization, although *S. aureus* can be present on other sites such as the skin or perineum. Dissemination of *S. aureus* among humans and from humans to food can occur through direct contact, indirectly through skin fragments, or through respiratory tract droplet nuclei (Seo and Bohach 2007).

Now-a-days, most sources of staphylococcal food poisonings are traced to humans who contaminate food during preparation. In addition to contamination by food preparers who are carriers, *S. aureus* may also be introduced into food by contaminated equipment used in food processing such as meat grinders, knives, storage utensils, cutting blocks and saw blades. A survey over 700 foodborne disease outbreaks revealed the following conditions most often associated with food poisoning: (i) inadequate refrigeration; (ii) preparation of foods far in advance; (iii) poor personal hygiene, e.g. not washing hands and instruments properly; (iv) inadequate cooking or heating of food; and (v) prolonged use of warming plates when serving foods (Bryan 1976).

To reduce the incidence of staphylococcal food poisoning, the aim will be to reduce initial load of *S. aureus* in a food by proper selection of raw materials and ingredients, sanitation of the food environments, and proper personal hygiene among the food handlers. Where possible, the products should be heat-treated to ensure killing of the live cells. Post-preparative contamination and temperature abuse should be avoided (Ray 2001).

2.2.4. Enterobacteriaceae

The term Enterobacteriaceae is derived from the Latin word enterobacterium, meaning an intestinal bacterium. The type genus for the family is *Escherichia* (Brenner 1984). The family Enterobacteriaceae includes a large biochemically and genetically related group of bacteria of heterogeneous ecology, pathogenic potential and host range. The organisms are facultative anaerobic, gram-negative straight rods of 0.3-1.5 µm in diameter that ferment glucose giving rise to acid and often gas. They are oxidase-negative and all, except *Erwinia chrysanthemi*, contain a common antigen. All the members of Enterobacteriaceae are catalase-positive, with a few exceptions, notably *Shigella dysenteriae*. They are

non-spore forming, non-acid fast and non-halophilic but tolerate the presence of bile salts (Brenner 1984).

Enterobacteriaceae are distributed worldwide. They are found in soil, water, fruits, vegetables, grains, flowering plants and trees, and in human and animal intestine (Brenner 1984). They are associated with many types of human infections, including more than 70% of urinary tract infections and nearly 50% of cases of septicemia. Until the 1940s, *Salmonella* and *Shigella* were considered to be the only gastrointestinal food or waterborne pathogens. In the late 1940s, enteropathogenic serotypes of *E. coli* were recognized as gastrointestinal pathogens, causing diarrhoea and vomiting in infants, and subsequently in adult volunteers.

Traditionally the group (designated as coliforms) has been chosen as an indicator of faecal pollution. These organisms are capable of fermenting lactose in the presence of bile at 37 °C. This group includes not only most strains of *Escherichia coli* but also organisms such as *Citrobacter* and *Enterobacter* which are not predominantly of faecal origin. The faecal coliforms, a more restricted group of organisms, are those coliforms which can grow and produce gas at 44-45 °C in suitable selective media (ICMSF 1978). One criticism of using coliforms and faecal coliforms is that their absence could give a false reassurance of safety when lactose-negative organisms predominate. The lactose-negative organisms include not only *Salmonella* and *Shigella*, but also enteropathogenic strains of *E. coli* itself such as O124. For this reason, tests for the whole of Enterobacteriaceae are increasingly being used. The Enterobacteriaceae includes even more genera of non-faecal origin than the coliforms, such as species of *Erwinia* and *Serratia* which are predominantly plant-associated. So, Enterobacteriaceae counts are used more generally as an indicator of hygienic quality rather than of faecal contamination and, therefore, say more about general microbiological quality than possible health risks posed by the product (Adams and Moss 1995).

Some important foodborne pathogens of this group, namely *E. coli, Salmonella* and *Shigella* are reviewed here.

2.2.5. *Escherichia coli*

Although *E. coli* was first isolated from children's faeces and described by Theodor Escherich in 1885, the first recognition of it as a cause of gastroenteritis in infants came in the early 1940s. Its common occurrence in faeces, ready culturability, generally nonpathogenic character, and survival characteristics in water led to the adoption of *E. coli* as an indicator of faecal contamination and the possible presence of enteric pathogens such as *Salmonella typhi* in water. This usage has been transferred to foods where greater circumspection is required in interpreting the significance of possible results (Adams and Moss 1995).

Until 1982, strains producing diarrhoea were classified into three types, viz. enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterotoxigenic *E. coli* (ETEC). However since 1982, *E coli* serotype 0157:H7, an enterohaemorrhagic type (EHEC) has been recognized as a cause of a number of outbreaks. The fifth type, enteroadherent *E. coli* has been implicated largely on epidemiological grounds. Its role in diarrhoea is under investigation (Adams and Moss 1995).

Cells of *E. coli* are gram-negative, catalase-positive, oxidase-negative, fermentative, short, non-spiral curved rods. *E. coli* is a typical mesophile growing from 7 to 50 °C with an optimum around 37 °C, although there have been reports of some ETEC strains growing at temperatures as low as 4°C. It shows no marked heat resistance, with *D*-value at 60°C of the order of 0.1 min, and can survivere frigerated or frozen storage for extended periods. A near-neutral pH is optimal for growth. Growth at pH 4.4 is possible under otherwise optimal conditions (Adams and Moss 1995).

The minimum a_w for growth is 0.95. Effect of 7.5 g sorbic acid l^{-1} (pH 5) on *E. coli* was reported by Doell (1962). Minimum inhibitory concentrations of 50-100 μg sorbic ml^{-1} (pH 5.2-5.6) and 50-1200 μg benzoic acid ml^{-1} (pH 5.2-5.6) were cited by Lueck (1980).

Symptoms of *E. coli* gastroenteritis include mild to severe diarrhoea. In severe cases, dehydration, prostration and shock may accompany the diarrhoea. Not all individuals show symptoms, but those who develop the symptoms shed the organisms in faeces after recovery (Doyle and Padhye 1989). EPEC strains typically cause infant diarrhoea in many tropical and developing countries, causing high mortality. ETEC strains cause travellers diarrhoea. Ingestion of 10^6-10^9 viable cells ml^{-1} by adults is necessary for the symptoms to occur within 24-72 h (Ray 2001).

Among 20 human and cattle isolates of *E. coli*, 80% were resistant to at least one antibiotic. Seventy percent were resistant to streptomycin, 65% to sulfonamide, 50% to tetracycline, 25% to ampicillin, 20% to sulfonamide/trimethoprim, 10% to chloramphenicol, 8% to cephalothin and 5% to gentamicin (Farina *et al.* 1996). Antibiotic resistance of 80% level for tetracycline, chloramphenicol, streptomycin and sulfonamide in some *E. coli* strains from mastitis infections in cows was reported by Teuber (1999).

The most important factor in the prevention of gastroenteritis in humans by pathogenic *E. coli* is to develop effective sanitation in water supplies, and treatment and disposal of sewage. Good personal hygiene and reheating of food before consumption should be practiced (Ray 2001).

2.2.6. *Salmonella*

Most salmonellae are regarded as human pathogens, though they differ in characteristics and severity of the illness they cause. Typhoid fever is the most severe, and consequently was the earliest salmonella infection to be reliably described. The typhoid bacillus was first observed by Eberth (1880). The paratyphoid bacilli, responsible for the clinically similar condition, paratyphoid fever, were first isolated by Achard and Bensaud (1896), and confirmed as culturally and serologically distinct from the typhoid bacilli by Schottmüller (1901). The genus *Salmonella* was created by Lignières in 1900 and named in honour of D.E. Salmon, the American veterinary pathologist who first described *Salmonella cholerae-suis* (now known as *Salmonella enterica* serovar Choleraesuis) isolated from swine suffering from hog cholera (Salmon and Smith 1885). The taxonomic nomenclature of the genus is rather different from that of other genera. On the basis of DNA-DNA hybridization, the genus *Salmonella* is now recognized to contain a single species, *S. enterica*, which comprises seven subspecies. Several classification schemes are currently used to clarify the biochemical and serological diversity within the *Salmonella* group (Chikami *et al.* 1985). In 1941 the scheme contained 100 serotypes, and the number has since risen to the current level of around 2000. The most recent proposal to introduce some taxonomic rectitude is to use the non-italicized serovar name after the species name so that *S. typhimurium* becomes *S. enterica* subsp. *enterica* serovar Typhimurium (Adams and Moss 1995).

Salmonellae are gram-negative, nonsporulating, facultative anaerobic rods (typically 0.5 μm x 1-3 μm) which are catalase-positive, oxidase-negative and generally motile with peritrichous flagella. They form gas while growing in media containing glucose (D'Aoust 1989). Generally, they ferment dulcitol but not lactose, utilize citrate as carbon source, produce hydrogen sulphide, decarboxylate lysine and ornithine, do not produce indole, and are negative for urease.

Salmonella spp. consist of resilient micro-organisms that readily adapt to extreme environmental conditions (D'Aoust and Maurer 2007). Growth occurs at temperature ranging from 5 to 47 °C with an optimum of 35-37 °C. Salmonellae are heat-sensitive and are readily destroyed by pasteurization temperatures (Adams and Moss 1995). Optimal pH for growth lies between 6.5 and 7.5 with possibilities

for growth at pH values ranging from 4.5 to 9.5 and slow death at more extreme conditions (Bryan *et al.* 1979). The growth of *Salmonella* is generally inhibited in the presence of 30-40 g sodium chloride l^{-1}. A higher temperature facilitated initiation of growth in the medium of greater salinity; high concentrations of sodium chloride protracted the lag period and decreased the rate of growth. Foods having a_w values of < 0.93 do not support the growth of salmonellae (D'Aoust 1989). Similar results were reported in a study of 23 strains of *Salmonella* grown at 10-30 °C in the presence of 20-80 g sodium chloride l^{-1} (Alford and Palumbo 1969). Inactivation of *Salmonella* by 7.5 g sorbic acid l^{-1} (pH 5.0) was reported by Doell (1962). Park and Marth (1972) found that 3 g sorbic acid l^{-1} (pH 5.0) inactivated *S. typhimurium* within 12 h in nutrient broth at 37 °C.

Salmonella has been recognized for its ability to harbour resistance to multiple antimicrobials (Besser *et al.* 1997; Breuil *et al.* 2000; CDC 1997; Cohen and Tauxe 1986). Bajaj *et al.* (2003) reported a very high level of resistance among 66 poultry egg isolates, particularly against penicillin (96.9%), vancomycin (83.3%), erythromycin (81.8%) and rifampicin (80.3%), a moderate resistance against trimethoprim (42.4%), chloramphenicol (28.7%) and streptomycin (24.2%), and a low resistance against gentamycin (3.0%), kanamycin (3.0%), furazolidone (16.6%) and tetracycline (9.0%). In another study on 91 *Salmonella enteritidis* isolates from broiler carcasses, food, human and poultry-related samples originated from South of Brazil, a high resistance was found against sulphonamides (75.8%) and nitrofurantoin (52.8%). Lower levels of resistance were found for tetracycline (15.4%), streptomycin (7.7%), nalidixic acid (7.7%), gentamicin (5.5%), norfloxacin (3.3%), trimethoprim (3.3%), cafalotin (2.2%), ampicillin (1.1%) and chloramphenicol (1.1%). In a study by the US Food and Drug Administration (FDA) on 502 *Salmonella* isolates from food samples, 247 (49.2%) were resistant to one or more antimicrobials of which 170 (68.8%) were resistant to one antimicrobial agent, 33 (13.4%) to two, 25 (10.1%) to three, 7 (2.8%) to four, 8 (3.2%) to five, and 2 (0.8%) each to six and seven antimicrobials (Kiessling *et al.* 2002). Study in two turkey processing plants in USA revealed that most *Salmonella* isolates were resistant to tetracycline, gentamicin, sulphamethoxazole, streptomycin, ampicillin, chloramphenicol and kanamycin (Olah *et al.* 2004). Similar effects have been reported by other researchers (Manie *et al.* 1998; Threlfall *et al.* 1997). Mayrhofer *et al.* (2004) reported highest resistance rate (42%) of *Salmonella* for nalidixic acid, followed by tetracycline (33%), streptomycin (27%), ampicillin, chloramphenicol (17%) and ciprofloxacin (9.6%). Of 52 *Salmonella* isolates from raw chilled retail poultry meats, 25 (48.0%) were resistant to one antibiotic, 5 (9.6%) were resistant to two, 4 (7.7%) were resistant to three, 6 (11.5%) were resistant to four, and 5 (9.6%) were resistant to five antibiotics. Two (3.8%) of the isolates were resistant to up to nine of the antibiotics tested. Fifty-one (98%) of the isolates were resistant to novobiocin, 18 (34.6%) to streptomycin, and 14 each (26.9%) to tetracycline and oxytetracycline (Jain and Chen 2006). In another study in USA on 208 *Salmonella* isolates recovered from imported foods, 23 (11%) isolates were resistant to at least one antimicrobial, and 7 (3.4%) to three or more antimicrobials. Resistance was most often observed to tetracycline (9%), followed by sulphamethoxazole (5%), streptomycin (4%), nalidixic acid (3%) and trimethoprim/sulphamethoxazole (2%). All the *Salmonella* isolates from betel leaves were sensitive to cotrimoxazole, and 97% of the isolates were sensitive to chloramphenicol, imipenem, ciprofloxacin, ceftriaxone and neomycin. Multidrug resistance (against 5-18 antibiotics) was common, particularly for nalidixic acid (65.8%), cephalothin (68.4%), cefoperazone (57.9%), sulphamethizole (52.6%), furazolidone (65.8%), kanamycin (68.4%), doxycycline (50.0%) and cefotaxime (44.7%) (Singh *et al.* 2006). For foodborne salmonellosis, an individual generally has to consume about 10^5-10^6 cells. However, there are some virulent strains where ingestion of fewer cells can cause the disease. Following ingestion of the pathogen, symptoms appear within 8-42 h. The general symptoms are abdominal cramps, diarrhoea, nausea, vomiting, chills, fever and prostration. It can be fatal to the sick, infants and the elderly (D'Aoust 1989).

Salmonellae are now established as one of the most important causes of foodborne illness worldwide. In Europe, in 1889, the annual incidence of salmonellosis was around 50 per 100,000 inhabitants. In USA, the incidence of salmonellosis continues to increase at a high rate: between 1969 and 1976 the average number of foodborne outbreaks was about 37 per year, while between 1983 and 1987 the number was over 68. Foods of animal origin including beef, chicken, turkey, pork, eggs, milk and products made from them have been associated with a large number of *Salmonella* outbreaks. Salmonellae have also been isolated from many foods of plant origin (due to use of sewage as fertilizer or washing products with polluted water), sea food, fin fish and crustaceans (Bean and Griffin 1990; Hatha and Lakshmanaperumalsamy 1997). *Salmonella* has frequently been implicated in foodborne outbreaks of illness with poultry meat being highlighted as a significant source of this pathogen (Bryan and Doyle 1995; Cloak *et al.* 1999).

Newborns, infants, the elderly and immunocompromized individuals are more susceptible to *Salmonella* infections than are healthy adults (D'Aoust 1989). Recent evidence suggests that 1-10 cells can constitute a human infectious dose (D'Aoust *et al.* 1985; Kapperud *et al.* 1990). Acute symptoms are nausea, vomiting, diarrhoea, fever and headache. Since birds, insects, handlers of infected food can all contaminate foods directly or indirectly, potential food vehicles for *Salmonella* are numerous.

Meat, milk, poultry and eggs are primary vehicles; they may be undercooked allowing the *Salmonella* to survive or these may cross-contaminate other foods which are consumed without further cooking.

Proper cooking of foods (minimum to pasteurization temperature and time such as 71.7 °C for 15 s or equivalent) and prompt cooling are important to control *Salmonella* infection. Prevention of cross-contamination of foods through cutting boards, equipment, utensils and hands should be practiced. Proper sanitation in the food environment and good personal hygiene can reduce the incidence. Refrigerated foods should be properly reheated before consumption (D'Aoust 1989). New knowledge that a single *Salmonella* cell can be infectious emphasizes the need for a greater stringency in food quality assurance programmes (D'Aoust *et al.* 1985; Adams and Moss 1995).

2.2.7. *Shigella*

The genus *Shigella* was discovered as the cause of bacillary dysentery by Shiga (1898). The genus contains four species which are serologically grouped based on their O-antigens, including *S. dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C) and *S. sonnei* (group D). Only humans and some primates are their hosts. The organisms are either transmitted directly through faecal-oral routes or indirectly through faecal-contaminated food and water. *S. dysenteriae* has been responsible for epidemics of severe bacillary dysentery in tropical countries, but is now rarely encountered in Europe and USA where *S. sonnei* is more common. *S. sonnei* causes the mildest illness, while that caused by *S. boydii* and *S. flexneri* is of intermediate severity (Adams and Moss 1995).

Shigellae are members of the family Enterobacteriaceae. They are non-motile, non-sporeforming, Gram-negative rods which are catalase-positive (with the exception of Shiga's bacillus, *S. dysenteriae* serotype 1), oxidase-negative and facultative anaerobes. They produce acid but no gas from glucose. The strains grow between 7 and 46 °C, with an optimum at 37 °C (Adams and Moss 1995). The cells survive for days under different physical and chemical stresses. These include refrigeration, freezing, 50 g sodium chloride l^{-1} and pH 4.5. They are killed by pasteurization. *Shigella* may survive in water up to 120 days. However, many food ingredients such as mayonnaise, vinegar, salt and sodium benzoate may be inhibitory. The strains are able to multiply in many types of food when stored at the growth temperature range (Smith 1987).

Isolation of *Shigella* from foods is not as facile as that from other sources. The physiological state of shigellae present in the food is a contributing factor in the successful recovery of this pathogen. *Shigella* may be present in low numbers or in a poor physiological state in suspected food samples. Under these conditions, special enrichment procedures are required for successful detection of *Shigella* (Andrews 1989).

Foodborne shigellosis is a neglected area of study on a global scale (Lampel and Maurelli 2007). The symptoms are the consequence of both the invasiveness of epithelial mucosa and the enterotoxin, and include abdominal pain, diarrhoea often mixed with blood, mucus and pus, fever, chills and headache. Generally, children are more susceptible to the disease than adults (Smith 1987). The infective dose is very low, about 10-1000 cells per person. Following ingestion of a contaminated food, the symptoms occur within 12 h to 7 days. In cases of mild infection, the symptoms last for about 5 or 6 days, but in severe cases the symptoms can linger for 2 to 3 weeks (Ray 2001).

Antibiotic therapy reportedly reduces the length of illness and of the carrier state for shigellae. However, wide use of antibiotics has resulted in selection of resistant strains and their resistance determinants. The best preventive measures for foodborne acquisition would be good personal hygiene and health education for food handlers. Proper treatment (chlorination) of water and sanitary disposal of sewage would prevent some foodborne as well as waterborne outbreaks of shigellosis (Doyle *et al.* 1985).

2.2.8. Occurrence of pathogenic bacteria in food

In spite of its undoubted value in nutrition, food has a long association with the transmission of disease. Regulations governing food hygiene can be found in numerous early sources such as the Old Testament, and the writings of Confucius, Hinduism and Islam. Such early writers had at best only a vague conception of the true causes of foodborne illness, and many of their prescriptions probably had only a slight effect on its incidence (Adams and Moss 1995). Even today, despite our increased knowledge, 'foodborne disease is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity'. Foodborne disease has been defined by the WHO as 'a disease of infectious or toxic nature caused by, or thought to be caused by, the consumption of food and water'.

The cause of foodborne gastrointestinal disorders can be broadly divided into three groups: (a) from the consumption of food and water containing viable pathogenic micro-organisms or their preformed toxins; (b) from the ingestion of pathogenic algae, parasites and their preformed toxins through food; and (c) for reasons other than viable pathogens or their toxins including natural toxic chemicals in food, biological amines, allergic components, nutritional disorder and overeating (Adams and Moss 1995).

Steadily changing urban life style triggers the continuous rise in foodborne bacterial diseases. There is an increase in the number of woman working outside to make their living. Hence, eating outside has become more of a compulsion for the members of the metropolis family. The convenience of getting RTE food at work place or near the home makes many to eat outside (Kakar and Udipi 2002). The various steps involved in the preparation and distribution of these foods provide ample scope for contamination with pathogenic and spoilage micro-organisms. As a majority of these foods are cereal- or pulse-based and involve restricted heat treatments, the commonly encountered contaminants are mainly *Bacillus* species. In a study on eight market samples of idli, none was contaminated with *B. cereus* though other species of *Bacillus* were present (Varadaraj *et al.* 1992). In 25 samples of traditionally cooked pulses obtained from local markets and different household communities, *B. cereus*

was present in 32% of samples (Shah *et al.* 1996). In a study comprising 10 samples of soya bean wadi and 20 samples of pulses from different retail shops and food establishments in and around Bareilly in the State of Uttar Pradesh in India, *B. cereus* was confirmed in 20% percent of each type of samples (Meena *et al.* 2000). Earlier investigations of neutral-to-alkaline legume-fermented foods, e.g. tempe, revealed the presence of considerable levels of potentially objectionable micro-organisms such as *B. cereus*, *S. aureus* and members of Enterobacteriaceae (Samson *et al.* 1987). In dawadawa, the presence of *B. cereus* was observed by Antai and Ibrahim (1986). Out of the 15 market samples of kinema tested, 5 samples containing > 10^4 cfu of *B. cereus* g^{-1} and 2 samples containing > 10^5 cfu of *E. coli* g^{-1} were found (Nout *et al.* 1998). Han *et al.* (2001) reported the presence of *B. cereus* and low numbers of *C. perfringens* in their study on 23 samples of sufu.

Though literature on the occurrence and subsequent outbreak of foodborne bacterial pathogens in the traditional legume-based fermented foods are scanty, ample information is there on other foods which substantiate that in recent years, foodborne diseases of microbial origin have become the number-one food safety concern among consumers and regulatory agencies. This trend is also true for other developed and developing countries (Garvani 1987).

In a food survey conducted in the Netherlands, te Giffel *et al.* (1996) reported *B. cereus* to be present in 48% over 200 food samples tested, with contamination level ranging from 10^2 to 10^6 cfu g^{-1} (or ml^{-1}). *B. cereus* was the cause of 33% of the total cases of food poisoning (excluding virus) in Norway during 1988-93, 47% in Iceland during 1985-92, 22% in Finland in 1992, 8.5% in The Netherlands in 1991 and 5% in Denmark during 1990-92 (Granum and Lund 1997; Schmidt 1995). Much lower numbers have been reported before from other countries, such as England and Wales (0.7%), Japan (0.8%), USA (1.3%) and Canada (2.2%) (Kramer and Gilbert 1989). In the USA from 1983 to 1987, an average of 479 foodborne disease outbreaks were reported each year, involving 18,336 individuals. However, on average, only 38% of the outbreaks involving about 10,908 individuals were confirmed. Out of these outbreaks, bacterial pathogens caused about 66% of the outbreaks affecting 92% of the cases and 96% of the fatalities (Bean *et al.* 1990). Several factors may be involved in the high incidence caused by pathogenic bacteria: many pathogenic bacteria are found in the raw materials of animal and plant origin, many are present in the food environments, many grow very effectively in different foods, and many are not killed by the conditions used for processing different foods (Ray 2001).

Although many pathogenic bacterial species and viruses have been implicated in foodborne disease outbreaks, there are some that have occurred at higher frequencies than others. Among the two most common pathogens associated with foodborne intoxication from 1983 to 1987, the number of outbreaks as well as deaths was higher for *C. botulinum*, but the total number of cases was much higher for *S. aureus*. Among the enteric pathogens causing infection, the largest number of outbreaks, cases and fatalities resulted from the foodborne infections caused by *Salmonella* spp. Toxicoinfection outbreaks and number of cases were higher for *C. perfringens* than for *B. cereus*. Of all the three types of foodborne diseases, *Salmonella* was associated with the highest number of outbreaks, affecting the largest number of individuals and causing the most deaths (Bean *et al.* 1990).

The following information gives a quick glimpse of the microbiological quality of different foods. Anand and Singh (1987) analysed 102 samples of infant foods for the presence of enterotoxigenic staphylococci. A wide variation was observed in their incidence with an average log count of 3.5 g^{-1}. Staphylococci were present in 57.8% of the samples. Among the staphylococci isolates, 30% produced enterotoxin. Chyan *et al.* (1989) found coliforms and *E. coli* in 31% and 9%, respectively, of the 606 rice products. In the study by Fang *et al.* (1997), the isolation rates of *B. cereus* in regular instant cereals and cereal-mix were 26% and 38%, respectively. Coliforms were isolated from 2.7% of regular instant cereal products as well as from 7.4% of cereal-mix products. Kakar and Udipi (1998), in their studies

on microbiological quality of fried RTE foods sold in Mumbai city, found that 11% samples of samosa and batatawada, and 45% samples of patra had staphylococcal counts of more than 3 logs. Coagulase-positive *S. aureus* was detected in three samples of samosa and two of patra. *Salmonella, Shigella* and *Yersinia enterocolitica* were not detected in any of the samples.

Fang *et al.* (1999) analysed the microbiological quality of 320 samples of vegetarian food purchased from local markets in China. The incidence of *E. coli* and coliforms in these vegetarian food products were 28% and 33%, respectively, while 18% and 3% of the samples were found to contain *S. aureus* and *B. cereus*, respectively. The samples made from soya bean showed the highest detection rate (74.5%).

Mukhopadhyay *et al.* (2002) evaluated the microbiological quality of street-vended sliced papaya (*Carica papaya*) in Kolkata. In their study, the total aerobic plate count ranged from 3.3 to 6.52 log cfu g^{-1} with an average of 5.96 log cfu g^{-1}. Coliforms were detected in 70% of the samples with an average load being 13.5 g^{-1}. The presence of *E. coli* was confirmed in 48% of the samples positive for coliforms. *Salmonella* and *Vibrio cholerae* were detected in one sample each, and low levels of coagulase-positive *S. aureus* were detected in 17% of the samples.

Altug and Bayrak (2003) studied the microbiological quality of caviar from Russia and Iran. The relevant figures that were detected among 68 samples are as follows: standard plate count varied from 10^3-10^6 cfu g^{-1}, coliforms varied from $<10^1$-10^4 cfu g^{-1}, yeasts varied from 10^1 to 6×10^5 cfu g^{-1}, *E. coli* varied from $<10^1$-10^2 cfu g^{-1}. Only in one sample *S. aureus* was detected as 5×10^2 cfu g^{-1}.

Hanashiro *et al.* (2005) studied the occurrence of some foodborne bacterial pathogens in 40 popular street foods available in a restricted area in São Paulo of Brazil and concluded that 35% of the samples were unsuitable for consumption according to the microbiological criteria. Mankee *et al.* (2005) studied the microbiological quality of 196 samples of 'bara', 'channa', condiments/spices and RTE 'doubles' sold in Trinidad. *E. coli* was detected in 0%, 7.1%, 49% and 34.2%, respectively, of the samples. Staphylococci were isolated from 53.1%, 36.2%, 65.8% and 62.8%, respectively, of the samples, and *B. cereus* was recovered from 11.2%, 43.4%, 51% and 44.9%, respectively, of the samples.

2.2.9. Behaviour of foodborne bacterial pathogens during fermentation

Bacteria become acid-stressed at low pH levels, but can survive this stress when they have previously been exposed to a less severe acid shock by the induction of an acid tolerance response. Acidification is a common method of food preservation, such as pickling and fermentation (Browne and Dowds 2002). *B. cereus* grew to more than 10^7 cfu ml^{-1} in the mageu base, which was not fermented by lactic acid bacteria, and it was implied that, on its own, *B. cereus* could grow in maize porridge to potentially toxic levels but was reduced to smaller numbers within 24 h. The inhibition of *B. cereus* was achieved from 10^6 cfu ml^{-1} to 10^2 cfu ml^{-1} in the mageu base inoculated with both the starter culture and *B. cereus* after 24 h-fermentation (Byaruhanga *et al.* 1999; Svanberg *et al.* 1992).

Güven and Benlikaya (2005) found that in the boza base inoculated with both the starter culture of lactic acid bacteria and *B. cereus* and in control boza base to which no starter culture was added, the *B.cereus* count dropped to 1 log cfu ml^{-1} after 72 h-fermentation. In this study, they observed a pH of < 4.9 after 12 h of fermentation in all batches of fermentation of boza in which *B. cereus* number gave a sharp decrease, and this finding is very well correlated with the records of Goepfert and Kim (1975) and Hancioglu *et al.* (1999). Nout *et al.* (1987) have shown that *B. cereus* grew rapidly to 8-9 cfu g^{-1} tempe made from non-acidified soya beans. Hancioglu *et al.* (1999) found that *E. coli* 0157:H7 was not inhibited and survived during 32 h-fermentation at pH 3.7. However, *S. typhimurium* and *S. aureus* were inhibited after 12 h of fermentation at pH < 4.5.

The fate of *B. cereus* introduced in the lactic acid fermentation at different stages was investigated by Wong and Chen (1988). The growth of lactic acid bacteria was not affected by *B. cereus*, however *B. cereus* increased rapidly to about 10^8 cfu ml^{-1} from initial level of 10^4 cfu ml^{-1} when cells were added at the beginning of growth of lactic acid bacteria. However, it was inactivated slowly when added after 24 h and rapidly when added after 72 h of lactic acid bacterial growth.

During tempe manufacture, *S. aureus* was able to survive in newly started soya bean soaks (final pH 4.7), whereas it died during soaks with accelerated souring (final pH 4.0). Enterotoxin levels were highest after 48 h-fermentation (Nout *et al.* 1988). Metaxopoulos *et al.* (1981) reported that in Italian type dry sausage produced using 10^5 cfu g^{-1} *Lactobacillus* sp., *S. aureus* increased by 1.5-log and fermentation with 10^4-10^5 lactic acid bacteria g^{-1}, it is increased by 2.5-log under the same fermentation conditions. Turantas (1991) found out that in sucuks with starter culture, the count of *S. aureus* dropped below the detectable limit starting from the sixth day.

The inhibitory effect on *S. typhimurium* and *E. coli* of prolonged incubation (0-48 h) by finger millet (*Eleusine coracana*) flour fermented for varying time periods was tested by Antony *et al.* (1998). Glass *et al.* (1992) studied the fate of *E. coli* 0157:H7 in fermented dry sausage. They inoculated a commercial sausage batter with 4.8×10^4 *E. coli* 0157:H7 g^{-1} and fermented to pH 4.8 and dried until the moisture-protein ratio was < 1.9 : 1. The sausage chubs were then vacuum packaged and stored at 4°C for 2 months. The organisms survived but did not grow during fermentation, drying or subsequent storage at 4 °C and decreased by about 2-log cfu g^{-1} by the end of storage. Survival of *E. coli* 0157:H7 during the fermentation of Datta and Awaze, traditional lactic acid fermented Ethiopian condiments, was tested by Tsegaye *et al.* (2004). When fermenting Datta or Awaze were initially inoculated at low inoculum level (10^3 cfu g^{-1}), the test strains were not recovered after 24 h of fermentation. At higher initial inoculum level (10^6 cfu g^{-1}), however, the counts of the test strains in Datta at day 7 were less by about 1.5-log units than the initial inoculum level. In fermenting Awaze, all the test strains were completely eliminated after 7 days. The pH of the fermenting green and red Datta was reduced from 5.2 to 4.4 and that of Awaze dropped from 4.9 to 3.8 during this time. Tsegaye and Ashenafi (2005) studied the fate of *E. coli* 0157:H7 during the processing and storage of Ergo and Ayib, traditional Ethiopian dairy products. They found that when milk was inoculated with both lactic acid bacteria and *E. coli* 0157:H7 and fermented for 72 h, the pathogen increased up to 24 h till the pH was 3.5, however, decreased at 72 h when pH became 3.9. In absence of lactic acid bacteria, the pathogen increased steadily.

Tetteh *et al.* (2004) studied the survival and growth of acid-adapted and unadapted *Shigella flexneri* in a traditional fermented Ghanaian weaning food as affected by fortification with cowpea. They found that *S. flexneri* remained viable, but did not grow in porridge made from fermented corn dough of pH 4.07 held at 10 or 30 °C for 24 h.