19

Food Borne Diseases

While food is necessary for sustaining life, it could also be a cause of illness. There is a general misconception that if a food is 'natural', it must be 'safe'. Unfortunately the fact that many toxins occur in natural plant foods, falsifies this naïve view. Most of these endogenous toxins are in plant foods and a few in animal foods.

Toxins from Plants

Solanine of potatoes is one of the best known plant toxins. It is a steroid which occurs in potatoes and other members of solanaceae family (e.g., aubergine) and the highly poisonous nightshades. Normally potatoes contain 2–15 mg per 100 g (fresh weight).

When potatoes are exposed to light and turn green, the level of solanine can be as high as 100 mg per 100 g. It is mostly concentrated under the skin. Potato sprouts may contain even higher amounts. Solanine can cause abdominal pain and diarrhoea, if injested in large amounts.

Solanine is an inhibitor of the enzyme acetyl choline esterase, which is a key component of the nervous system. Ingestion of solanine have been reported to lead to signs of neurological damage.

As there is a general public awareness of the health hazards of eating green potatoes, the incidence of potato poisoning is low.

Solanine is **not lost** during normal cooking as it is insoluble in water and is heat stable.

Caffeine is a purine alkaloid. **Theobromine** is another important member of this group. These occur in tea, coffee, cocoa and cola beverages, which are regarded as stimulants.

But there are three good reasons for treating these as toxins. Firstly, these could never be regarded as nutrients, secondly they are addictive in nature and thirdly their physiological effects are dependent on the amount ingested.

Phenylethylamine found in chocolate brings on migraine headache in susceptible persons. Phenylethylamine and **serotinin** are vasopressor amines, which occur in plant foods, or are formed during fermentation in cheese and wine. Constriction of blood vessels, especially in brain is the usual effect of these amines. Hence there is a need to minimise the intake of these.

Hydrogen cyanide released from glycosides in foods can be toxic. When there is tissue damage of tapioca during harvest or preparation for cooking hydrogen cyanide is formed from amygdalin, a glucoside in tapioca. Therefore, tapioca varieties, rich in cyanogenic glucosides need to be fermented

in preparation to release HCN as a volatile gas and thus make it safe for consumption, as is done in West Africa in preparation of "Garri", a staple of that region.

In spite of this, chronic cyanide poisoning occurs in tapioca eating persons, due to habitual low level intake of cyanides. One of the common diseases in such conditions can lead to neurological degeneration and a form of blindness.

Lima beans also contain generous amounts of glycosides. It is difficult to cook lima beans so as to eliminate their toxicity; so bean varieties with low levels of glucosides are being produced by breeding.

Besides cyanogens, legumes contain *inhibitors* of trypsin and chymotrypsin. Peas, beans, soybeans and groundnuts contain protease inhibitors (the proteases are trypsin and chymotrypsin). The inhibitors in most legumes are inactivated by cooking except in soybeans, which have to be autocalved to achieve the same.

Some plants when consumed can cause food poisoning. Certain varieties of mushrooms are very poisonous and could even be fatal if consumed. Snakeroot poisoning could result from drinking milk from cows that have fed on this weed.

Lectins or **haemoglutinins** present in kidney beans are also toxins. The name haemoglutinin indicates that these toxins bind to the surface of red blood cells and cause them to clump. These are destroyed during cooking. There is massive breakdown of lectins when beans are germinated.

The vogue of eating raw foods poses a potential hazard, especially if the diet consists of *only* raw foods. The lectins are toxic in the region of 0.5 mg/kg.

Myristicin, a toxin occurs in significant amounts in nutmeg and smaller amounts in black pepper, carrots and celery. 10 g of nutmeg contain enough myristicin to produce initial euphoria, hallucinations and narcosis, just like a heavy dose of alcohol. Since nutmeg is used in very small amounts as a flavouring only, it only induces sleep. But it is not advisable to eat nutmeg flavoured foods in pregnancy.

Toxins from Animals

Tetrodotoxin is a toxin, which occurs in the organs (liver, ovaries) of the puffer fish. The muscles and testes of puffer fish is a popular delicacy in Japan. As the minimum lethal dose of tetrodotoxin lies between 1.5 and 4.0 mg. Great skill is needed by food handlers to separate the deadly parts of fish from edible ones. Expert cooks have to be strictly licensed, but fatalities occur regularly.

The toxin blocks movement of sodium across the membranes of nerve fibres, disrupting transmission of nerve impulses. A number of nervous symptoms develop leading to total paralysis and respiratory failure. Death occurs within 6 to 24 hours. No effective treatment has been found so far.

Other toxins find their way into shellfish such as mussels, cockles, clams and scallops due to a type of plankton, at certain times of the year. Some of these planktons are red coloured and proliferate in coastal waters. When these occur, these are called *red tides*. Hence, coastal communities have

avoided fishing during red tides. But in cooler parts such as Alaska and Scandinavia, the planktons are not coloured. Hence, routine checks are carried out on toxin levels of shellfish in these regions.

Sea food such as mussels and clams sometimes contain a poisonous alkaloid and could cause food poisoning symptoms.

A different type of poisoning occurs when fish from the family *Scrombridae* (including tuna, sardines, mackerel) are held above 10°C for some hours, due to formation of high levels of histamine from tryptophan in the muscle. This reaction is catalysed by the decarboxylase produced by bacteria present in the fish. High levels of histamine (100 mg/100 g) are produced before the putrefaction occurs. Susceptible persons suffer from headaches, palpitations, gastrointestinal upsets, skin flushes, and erethrema. The symptoms are relieved by taking antihistamine drugs. The residents of the areas where such poisonous plants and animals are found usually know about the hazard and guard against it.

Mycotoxins

Toxins produced by moulds are known as *mycotoxins*. There are over 150 mould species, which produce toxins when grown on foods. Of these, two important ones are **ergot** and **aflatoxin**.

Ergotism is a disease known since the Middle Ages when it occurred in epidemic proportions in Europe due to infected rye which was used as a staple food. When rye and other cereals are infected by the mould *Clariceps purpurae* or *ergot*, at one stage in its life cycle, it produces hard, purplish black masses of dormant cells called *sclerotia*.

These have the same size as a cereal grain. If the sclerotia are not removed by sorting before milling, these are milled with the cereal into flour. The sclerotia contain 20 different toxic alkaloids, *ergotamine* being the most abundant one. The effects of these alkaloids on the body are not well understood, but depend on the kinds and amounts produced by the moulds present. Symptoms include burning pains in hands and feet, loss of sensation in the limbs, followed by gangrenous withering, blackening and loss of the limbs. Simultaneously, there is mental derangement and gastrointestinal failure. Finally the victim dies.

The disease is rare now due to reduction of infected plants with the use of fungicides, application of modern drying methods to prevent post harvest growth of microorganisms and the use of mechanical grain cleaning methods, which separate heavy grains from the light sclerotia formed.

Of the three genera *Fusarium*, *Penicillium* and *Aspergillus*, which infect foods, especially cereals, *Aspergillus* is the most dangerous one.

A flavus produces aflatoxins, which are carcinogens. **Aflatoxin** B_1 is one of the most potent liver carcinogens known. Use of mouldy cereals containing 0.2 to 20 mg per kg aflatoxin have led to many fatalities in the tropics. The level of aflatoxin in staple foodstuffs has been found to be positively correlated to incidence of liver cancer.

Groundnuts and their products from the third world countries are potential sources of aflatoxin in the diet. When these contaminated feeds are used as cattle feed 1% of the aflatoxin is found in milk but not much comes through in the meat.

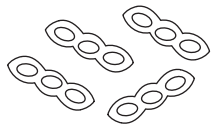

Fig. 19.1 Groundnuts

Aflatoxin can be easily detected and quantified by using a fluorimeter.

Production of aflatoxins can be prevented by discarding mouldy cereals and nuts. In traditional foods, which need ripening by moulds, pure starter cultures of safe mould strains are used instead of 'natural' inoculants. Prevention of mould contamination from factory walls, plant and machinery and employees is essential.

Toxic Agricultural Residues

Residues of pesticides which are toxic may enter the food when used in agriculture for killing weeds, insects and microflora. **DDT** a pesticide used for killing mosquitoes has been found in many foods in proportions far exceeding the safety threshold. Some of the other pesticide residues found in food include **dieldrin**, **lindane** (**BHC**) and **malathion**, which are used for spraying on fruits and vegetables to control insects and microflora. Many of these pesticides have been banned. But their use for many years has left an appreciable amount in the soil, which find their way through the crops into human diet (Table 19.1).

A second source of residue are **sterols**, used for increasing growth in animals raised for meat. Residues of these sterols may be present in meat of these animals. Some of these sterols have been shown to be carcinogenic.

The final type of agricultural residue are **antibiotics**. For example, **penicillin** is used to treat cows with masitis. Milk from treated cows must be discarded, but it may not be. The penicillin in milk can cause allergic reaction in sensitive persons. It will also interfere with manufacture of curd and cheese. **Antibiotics** are also used as growth promoters in animal feed. Animals fed antibiotic may develop resistant strains of bacteria, which may infect humans and cause untreatable disease. Therefore, the unscrupulous use of antibiotics in animals needs to be prohibited.

Poisoning by Chemicals

Toxic metals may reach our food from a number of sources. The most important sources include water used in food processing and cooking, soil on poorly washed vegetables, water pipes (lead), equipment, containers and utensils used for processing, storage and cooking of food.

TABLE 19.1 Common Pesticide Residues in Meat, Fish and Poultry[1]

Pesticide	Content (p.p.b.[3])	Intake (p.p.b.)	ADI[2] (p.p.b.)
DDT	<1	0.034	5
Alpha BHC (benzene hexachloride)	<1	0.011	
Gamma BHC (lindane)	<1	0.003	10
Malathion	<1	0.203	20
Polychlorinated biphenyls	2	0.008	

1. Based on survey done in U.S.A in 1980.
2. Acceptable Daily Intake recommended by FAO/WHO μg/kg/day.
3. Parts per billion.

Lead the use of lead piping and tank in water supply can lead to a higher level of lead, if water is soft. Lead contamination of beverages is restricted to illicitly produced alcoholic drinks, as also poorly glazed pottery used to store acidic beverages. Use of lead-based solder can be a source of lead contamination in canning. As most foods are subject to regulation of lead content, the likelihood of lead poisoning is not very common.

However, symptoms of chronic lead poisoning such as anaemia, poor I.Q. performance etc. may be found in children from congested city environment, living in dwellings that have peeling, deteriorated lead surfaces. They may eat paint chips, breathe air-borne dust particles released from paint before remodeling. In addition, food and water contribute to daily lead intake. The lead pigment used to print labels on soft plastic food packaging can also be a source, especially as families reuse the bag for food storage.

The tetraethyl lead in exhaust fumes may be an additional source of lead. For example, studies in U.S.A. showed that absorption of lead in children is eight times greater than adults and tends to accumulate in their bodies. The brain-damaging effects of lead poisoning in young children is associated with anaemia, fatigue, poor attention span and learning ability. Hence, there is a need for a vigorous plan of prevention to eradicate this serious problem.

Mercury Inorganic mercury compounds reach our food from two sources – cereal seed grains treated with antifungal mercury compounds, meant for planting, being mistakenly used as food and industrial pollution of coast water contaminating fish and other seafoods with alkyl mercury compounds. Recently, there was a case reported in Ootacumund, Tamil Nadu of metallic mercury poisoning due to contamination of water from the effluent in a factory manufacturing thermometers.

The symptoms of mercury poisoning are variable but all point to damage to the central nervous system. In all situations, children, being vulnerable, are more sensitive to the exposure.

Arsenic is widely distributed in nature and may contaminate chemicals used in food processing and thus finds its way into food. For example, the use of sodium phosphate contaminated with arsenic trioxide as stabilizer in milk powder led to the poisoning of 12,000 infants in Japan, who were fed the formula made from that milk powder; at least 120 died.

Therefore, it is important to check for arsenic contamination in food additives.

Cadmium Contamination of foods from cadmium can occur from three sources. The first is use of contaminated water (e.g., water from mine operation as occurred in Japan in 1960) for the irrigation of paddy. Second is use of cadmium plated components in food processing machinery. The third is zinc plating or galvanizing of containers used to store acid foods. Usually zinc contains some cadmium, which may get dissolved with zinc and enter food products.

All these metal contaminants **lead**, **mercury**, **arsenic** and **cadmium** are bivalent, similar in some ways to nutrient mineral like iron, calcium and zinc. If the diet is low in bivalent nutrient metals (calcium, iron) the contaminants may enter the vulnerable systems (nerves, kidney, bone marrow), accumulate and disturb their function. There is a need to monitor the environmental contamination of foods by these metals.

Food Poisoning by Bacteria

There are two chief kinds of food poisoning caused by bacteria:

(i) **Botulism** caused by the presence of toxin in the food produced by *Clostridium botulinum* and
(ii) **Staphylococcus poisoning** caused by a toxin produced in the food by *Staphylococcus aureus*.

Botulism *Clostridium botulinum*, is a spore forming, anaerobic microorganism found in the soil. Foods such as corn, beans, peas, meat, fish are likely to be contaminated with the spores of this organism. If these contaminated foods are not given adequate heat treatment during canning, these spores survive and the *Clostridium botulinum* multiplies in the can, as it is an anaerobic organism. During the process of growth, some strains of this organism produce a toxin in the food, which is a potent poison. If the food containing the toxin, is consumed without heating, it can cause paralysis of involuntary muscles and may ultimately lead to death due to respiratory failure. This effect is known as **botulism**. The toxin is, however, destroyed on heating. Any suspected food should, therefore, be boiled for at least 15 minutes before it is consumed. The incidence of botulism is, however, very rare as all commercially heated processed foods manufactured by reputable companies are always given approved heat treatment which is enough to kill pathogenic bacteria that may be present in the food. It is likely to occur if neutral foods are inadequately processed at home and consumed by families.

Staphylococcus Food Poisoning The causative organism of staphylococcus poisoning is *Staphylococcus aureus*. Staphylococcus contamination of the food may either be from human or animal sources. The nasal passage of many human beings, especially those with sinus infection contains many such *staphylococci*. Similarly, boils and infected wounds are potential sources. Cows

affected by a disease called *mastitis* could discharge *staphylococci* into the milk. Environmental conditions such as temperature (37°C), presence of an abundance of protein and starch, are conducive to growth of *staphylococci*. Some strains of *Staphylococcus aureus* can produce an enterotoxin. Enterotoxin is a toxin produced by an organism outside the cell-wall and can thus be produced in the food even when the microorganism is living. In contrast, endotoxins are produced inside the cell-wall and can only permeate the food or the body when the organism is killed.

Foods containing **staphylococcal enterotoxin** when consumed can cause salivation, nausea, vomiting, abdominal cramps and diarrhoea. Recovery takes about a day or two and mortality is very low. The time between the consumption of the food and the appearance of symptoms can range from 1–6 hours.

Precautions should thus be taken to prevent entry of *staphylococci* into foods if such food poisoning is to be avoided. Employees suffering from *staphylococcal* infections such as colds, boils should not be allowed to handle foods. Refrigeration of foods immediately after preparation prevents growth and formation of enterotoxin by *staphylococci.* Pasteurisation kills all the *staphylococci* that may be present in foods.

Food Infections

In contrast to food poisoning, food infection can be caused by organisms growing in food in large numbers. One such organism which has been known to cause symptoms very similar to the *staphylococcal* poisoning seen earlier is the *salmonella bacillus*. The various species of *Salmonellae* vary in their degree of infectiveness. Some like *Salmonellae enteritidis* can cause infection when a few organisms (about a million or so) are consumed whereas with other less infective species such as *Salmonellae pullorum* hundreds of millions of the organism would have to be ingested before they can cause infection.

The incubation period of *salmonella* infections is longer than that for staphylococcal poisoning, the period being usually between 12 and 24 hours.

The symptoms observed in **salmonellosis** are nausea, vomiting, abdominal pain and diarrhoea. The recovery is usually uneventful even though it may take two to three days. Some of the persons attacked by the organism after being cured become carriers of the microorganism.

People can be prevented from being infected by the disease by: (i) avoiding foods which are contaminated by the organism through diseased human beings and animals, e.g., eggs, which are broken in transit are also prone to contamination by the organism (ii) by preventing the growth of the organism by adequate refrigeration after the food has been prepared, and (iii) by pasteurising the food at such temperatures and times to kill all the pathogens present.

Other Infections

Besides salmonellae, other organisms such as the *streptococci* can also cause infections by growing in sufficient numbers in the food.

Foods can act as carriers of certain microorganisms causing diseases such as typhoid, paratyphoid, bacillary and amoebic dysenteries but none of these organism grow in the food as such. Usually, such contamination may occur due to poor handling after preparation.

It is essential, therefore, that in eating establishments and food processing factories, workers handling food do not suffer from communicable disease or are carriers of infections.

Points to Remember

Many toxins occur in plant and animal foods.

Toxins from plants include solanine (potatoes), caffeine (tea, coffee), phenylethylamine (chocolate), hydrogen cyanide (tapioca,/cassava) and trypsin inhibitor in legumes, lectins (kidney beans), myristicin in nutmeg.

Poisonous plants include some varieties of mushrooms and snakeroot.

Toxins from animals include tetrodotoxin in puffer fish, toxins in shellfish from planktons, poisonous alkaloids in muscles and clams etc. Histamine produced in certain fish by bacterial action is also toxic.

Mycotoxins prominent ones are sclerotia from ergots (a mould) in infested cereals and aflatoxins from infected groundnuts and cereals, both of these have deleterious effects on health.
Toxic agricultural residues include pesticide residues, sterol residues in meat and antibiotic residues in milk and meat

Poisoning by chemicals Lead, mercury, arsenic, cadmium enter human body through food, water and environmental contamination. These enter vulnerable body systems and disturb their function.
Bacterial food poisoning occurs due to bacterial toxins e.g., botulism and staphylococcus enterotoxin.
Food infections are caused by ingestion of large number of bacteria (salmonella and streptococci).

Knowledge of these can help us to prevent these.

Study Questions

1. What is botulism? How can it be prevented?
2. Discuss the steps to be taken during preparation and storage of prepared foods to prevent staphylococcus food poisoning.
3. What is food infection? How can it be prevented?

20

Food Laws and Food Standards

Most persons in India, spend more money for food than any other item. Dietitians and nurses at work and in their social contacts are constantly asked about the comparative merits of foods available.

As food accounts for a large part of the family budget (expenditure), the homemaker tries to be selective in her purchasing. She would like to compare the available products not only in quantity but also in its nutrient contribution. Many consumers are also concerned about the additives in foods and would like to know if these additives are safe and are necessary. Other persons may need to follow a diet, which is modified in terms of fat content, cholesterol content etc.

These and many consumer problems are solved through knowledge of laws that protect the quality of food supply as well as the requirement that advertisers make no false claims for their products. Finally, the consumers need information concerning how to select food wisely within the available food groups.

One of the most common problem of food marketed is adulteration. The consumers like to get maximum quantity for as low a price as possible. The sellers must meet the needs of the buyers to be able to sell. In addition, the sellers have to be able to make a profit to be able to exist. This is a vicious cycle. When the price of food production is higher, than the price which the consumer is prepared to pay, the seller is compelled to supply a food product of inferior quality. Thus, adulteration occurs.

Adulteration is defined as the process by which the *quality* of the product is reduced through addition of *baser* substance or removal of a *vital* element. For example, water may be added to milk to increase its volume *or* fat may be removed from it.

It was to check such malpractices that the first central act called the Prevention of Food Adulteration Act was passed in 1954 and came into force from June 1, 1955. The PFA pertains to food sold and defines what may be considered as adulteration. It requires that foods be pure, wholesome and honestly labeled.

Food Laws

The Government of India is fully alive to the possibilities of food being adulterated. It has, therefore, empowered several agencies and promulgated a number of acts and orders to counteract this menace. Agencies and institutions have also been created to lay down standards for the quality of foods. The manner in which the food is processed and packaged is also covered by a number of

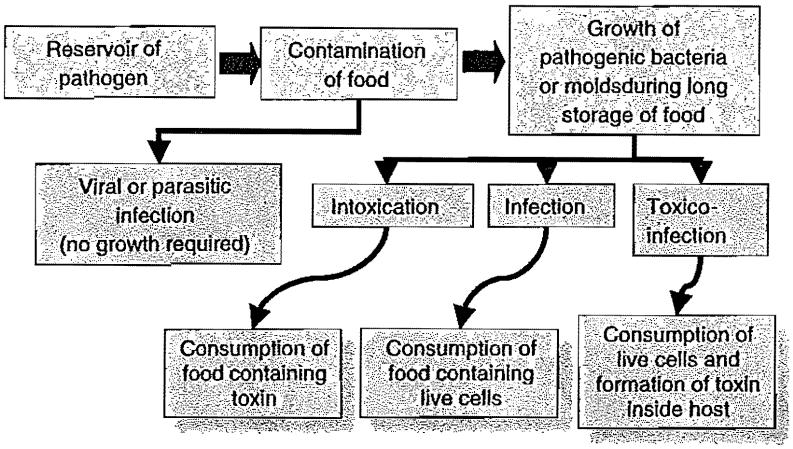

FIGURE 23.1 Sequence of events that lead to foodborne disease by bacteria and viruses.

Acceptance Quality of Food Due to Growth of Pathogens

Pathogenic viruses need viable host cells for growth; thus in prepared foods they cannot grow and they do not affect the food quality. Pathogenic bacteria can grow in many foods. When the environment is suitable, only a few viable cells present initially can reach a high level, maybe several millions per gram or milliliter. However, growth of some pathogens, even to a high level, may not alter the color, texture, and odor of a food (such as *Sta. aureus*). People can consume this food without suspicion and develop symptoms of a foodborne illness.

Sequence of Events in a Foodborne Disease

For a foodborne disease to occur, several events have to happen in sequence (Figure 23.1).[1,2] An understanding of these sequences is helpful in investigating the cause (the source and means of transmission) of a foodborne disease. It also helps in recognizing how the sequence can be broken in order to stop a foodborne disease. Initially, there has to be a source of a pathogen. Next, the pathogen has to contaminate a food. Consumption of the food contaminated with a pathogenic virus or parasites may lead to viral or parasitic infection. For bacterial pathogens (and toxicogenic molds) the contaminated food has to support growth and be exposed for a certain period of time at a suitable temperature to enable the pathogens to grow. However, for some potent pathogens (such as *Esc. coli* O157:H7) growth may not be necessary to cause a foodborne infection. For intoxication, the growth should reach a sufficient level to produce enough toxins so that when the food is consumed, the individual develops the symptoms. For bacterial infection, viable cells of a pathogen need to be consumed in sufficient numbers, which vary greatly with pathogens, to survive stomach acidity, establish in the digestive tract, and cause illness. In case of toxicoinfection, viable cells should be consumed either in very high numbers (for those that cannot multiply in the digestive tract, such as *Clo. perfringens*) or in reasonable numbers (for those that can multiply in the digestive tract, such as *Vibrio cholerae*), so that toxins released by them in the digestive tract can produce the symptoms.

CURRENT TRENDS

In 1997, a summary review on foodborne illnesses in the United States between 1988 and 1992 was published. At present, through FoodNet, a foodborne disease surveillance network of the regulatory agencies has been set up, which makes it possible to obtain available foodborne outbreak reports relatively quickly. In addition, a recent article from the CDC, based on a scientific approach, listed

the estimated foodborne illnesses for each currently known foodborne pathogens. This information is summarized here to evaluate the current trends of foodborne illnesses as compared to the past (between 1982 and 1987; presented in tables in this chapter).

Foodborne Disease Outbreaks During 1988 to 1992

Bean et al. (1997)[5] reviewed the data collected by CDC on reported foodborne disease outbreaks in the United States during 1988 to 1992. During this period (Table 23.7), like before (Table 23.1), the incidence of outbreaks, number of cases, and number of deaths declined, but were still much higher than other etiological agents. Like the previous period, the incidence of illnesses during this period were highest from foods of animal origin (Table 23.4) and occurred more frequently during May to October with the greatest during June to August. Similarly, as before (Table 23.5), homes (15.2%) and food services (35.7%) were the major sources of foodborne illness during this period. Among the pathogens the incidence was the highest with *Salmonella*, (65.4%; Table 23.8) and showed an increase of 10% over the previous period (55.1%; Table 23.3). Serovar Enteritidis was the predominant isolate. The frequency of incidence of outbreaks for the other pathogens remained very similar during both periods. During both periods, the three major contributing factors for the outbreaks remained essentially the same, namely improper holding temperature, poor personal hygiene, and inadequate cooking (Table 23.9). The frequency of contaminated equipment (including cross-contamination) increased slightly during this period.

A comparison of the results for the two 5-year periods indicates there is very little change in the foodborne disease outbreaks as factors or epidemiology associated with the outbreaks.

FoodNet

The Centers for Disease Control and Prevention (CDC), along with the Food Safety Inspection Service (FSIS) of the USDA, the federal Food and Drug Administration (FDA), and the eight Emerging Infections Program (EIP) sites developed the FoodNet Program in an effort to reduce foodborne diseases in the United States. The major objectives of FoodNet are to determine: (1) the frequency and severity of foodborne diseases; (2) the proportion of common foodborne diseases that result from eating specific foods; and (3) the epidemiology of new bacterial, parasitic, and viral foodborne pathogens. To obtain the information, FoodNet conducts active surveillance and related epidemiological studies. FoodNet can determine the effectiveness of the programs initiated by interagency National Food Safety Initiative to reduce foodborne diseases. One example is to study the

TABLE 23.7
Foodborne Disease Outbreaks, Cases, and Deaths by Etiological Agents During 1988 to 1992 in the United States

Etiological agents	Outbreaks		Cases		Deaths	
	No.	%	No.	%	No.	%
Bacterial	796	32.9	33,183	42.9	55	79.7
Viral	45	1.8	2401	3.1	6	8.7
Parasitic	17	<1.0	379	<1.0	0	0
Chemicals	143	5.9	927	1.2	4	5.8
Unknown	1422	58.7	40,487	52.3	4	5.8
Total	2423	≃100	77,377	≃100	69	100

[a] Death: *Clo. botulinum*, 11; *Salmonella*, 38; *Lis. monocytogenes*, 1; *Vib. cholerae*, 1; *Clo. perfringens*, 1; Hepatitis A, 4; paralytic shellfish, 2; scombroid, 1.

D. Enumeration of coliforms faecal coliforms and *E. coli* in foods using the MPN method

1. Application

The Most Probable Number (MPN) method is applicable to the enumeration of coliforms, faecal coliforms and aerogenic *Escherichia coli* in foods, food ingredients and water, including contact water from food manufacturing plants.

Note: This method is **not** intended to be used to isolate and enumerate *E. coli* serotypes associated with human illness, particularly the enterohemorrhagic serotype O157:H7. Many of the pathogenic serotypes do not give a positive faecal coliform reaction and therefore would not be detected and recovered by this method.

2. Description

The MPN procedure involves a multiple tube fermentation technique where three or more decimal dilutions of the sample are inoculated into tubes of broth medium and incubated at a specific temperature and for a specific time. The method is progressive; i.e., first determining the presence of coliforms in the tubes, then determining if these tubes also contain faecal coliforms, and then confirming whether *E. coli* is present. Based on the number of tubes indicating the presence / absence of the three groups of organisms, the most probable number present can be estimated from a standard statistical MPN table. The method has been shown to produce satisfactory results with naturally-contaminated foods and water for the detection of coliforms, faecal coliforms and aerogenic *E. coli.*

3. Principle

The terms "coliform" and "faecal coliform" have no taxonomic validity and, therefore, are only meaningful when expressed in terms of the analytical test parameters of medium, time and temperature of incubation.

Coliforms, faecal coliforms, and *E. coli* are considered "indicator organisms."

The presence of "indicator organisms" in foods processed for safety may indicate one of the following possibilities: 1. inadequate processing and/or post-processing contamination; and/or 2. microbial growth. The presence of faecal coliforms and *E. coli* may indicate faecal contamination; however, it must be understood that these microorganisms can survive and multiply in a variety of non-intestinal environments, including the processing plant. When assessing the presence of "indicator organisms" in a sample, **one must assess the results against the tolerance limits specified by government standards or guidelines, health agencies, or a laboratory's in-house specifications,** keeping in mind that established standards and guidelines are specifically linked to the method used to develop these standards.

As indicated in section 1, the presence of coliforms, faecal coliforms and aerogenic *E. coli* in food and water may be determined by means of the MPN procedure. Briefly, this method involves serially diluting out the target organisms in the sample, in 5-replicate aliquots, to extinction. The probable level of the target organisms is then statistically estimated from an MPN table.

Gas production is used as an indication of ability to ferment lactose from LST broth (presumptive coliform test); gas production from BGLB broth is considered confirmation of coliform presence;

gas production at 44.5 or 45° C from EC broth is used as confirmation of faecal coliform presence; and appearance of typical nucleated, dark-centred colonies with or without metallic sheen when positive EC broths are streaked onto L-EMB agar are indicative of *E. coli*. The typical colonies on L-EMB agar must be confirmed by further biochemical tests to prove the presence of *E. coli.*

4. Materials and special equipment

The media listed below (1 to 8) are commercially available and are to be prepared and sterilized according to the manufacturer's instructions.

Note: If the analyst uses any variations of the media listed here (either product that is commercially available or made from scratch), it is the responsibility of the analyst or Laboratory Supervisor to ensure equivalency.

1) Peptone Water (0.1% and 0.5%)

2) Aqueous Sodium Citrate (2.0%), tempered to 40-45°C

3) Lauryl Sulfate Tryptose (LST) broth

4) Brilliant Green Lactose 2% Bile (BGLB) broth

5) *Escherichia coli* (EC) broth or EC broth with MUG (4-methylumbelliferyl-ß-D-glucuronide)

6) Levine's Eosin Methylene Blue (L-EMB) agar or Endo agar

7) MacConkey agar

8) Nutrient Agar (NA) or other non-selective agar

9) Covered water baths, with circulating system to maintain temperature of 44.5°C and 45°C. Water level should be above the medium in immersed tubes.

10) Thermometer, calibrated and traceable

11) Incubator, 35°C.

12) Stomacher, blender or equivalent.

13) Control cultures (use ATCC cultures or equivalent): positive control(s): *E. coli* that is known to produce gas at 44.5 / 45° C and is capable of fermenting lactose to produce typical reactions on L-EMB agar; if using EC-MUG, a strain that is known to produce ß-glucuronidase EMB / IMViC negative control: *Enterobacter aerogenes* or an equivalent gram negative rod that does not produce "positive" reactions on EMB and is indole-negative, methyl red-negative, Voges-Proskauer-positive, and citrate positive. MPN broths negative control: *Salmonella berta* or an equivalent gram negative rod that is gas-negative in MPN broths and in the secondary EC broth

NOTE: Some strains of *E. aerogenes* will give false-positive reactions in the MPN broths (LST, BGLB and EC broths) by producing a small gas bubble. Therefore, use *S. berta* or an equivalent culture for these broths and *E. aerogenes* or an equivalent culture for EMB agar and IMViC tests.

14) pH meter capable of distinguishing to 0.1 pH units within the range of pH 5.0 to 8.0 or pH paper capable of distinguishing from 0.3 to 0.5 pH units, within the same range.

15) Supplies needed for confirmation (commercially available): The following supplies may be needed for confirmation; use A or B (see 7.9). The choice of further identification schemes (7.9.5) may require alternate media

A. IMViC media and reagents:

a. Tryptone (or tryptophane) broth Indole reagents (available commercially)

b. Buffered Glucose broth Voges-Proskauer test reagents (available commercially) Methyl red solution

c. Simmon's Citrate (SC) agar

B. Rapid Identification Kits or Systems (such as API, Vitek or equivalent)

5. Procedure

Each sample unit may be analyzed individually or the analytical units may be combined where requirements of the applicable sampling plan can be met. Carry out the test in accordance with the following instructions:

5.1. Handling of Sample Units

5.1.1. In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated (0-5°C) or frozen, depending on the nature of the product. Thaw frozen samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death.

5.1.2. Analyze sample units as soon as possible after their receipt in the laboratory. Shellfish must be analyzed within 24 hours of collection.

5.2. Preparation for Analysis

5.2.1. Have ready sterile peptone water.

5.2.2. Clean the surface of the working area with a suitable disinfectant.

5.2.3. Arrange LST broth tubes in rows of five and mark them identifying the sample unit and the dilution to be inoculated (Table II).

5.3. Preparation of Sample, Initial Set-up and Reporting- Raw and Processed Shellfish

5.3.1. For all shellfish, always use 0.5% peptone water for all dilutions.

5.3.2. Include only live animals in the sample for unfrozen shellfish. Select 10 or more animals to obtain a minimum of 200 g of meat and liquor.

5.3.3. Scrape off all extraneous growth and loose material from the shell and scrub the shellfish (including the crevices at the juncture of the shells) with a sterile stiff brush under running water of potable quality. Do no use faucets equipped with aerators. Drain shellfish in a clean container or on clean towels.

5.3.4. Disinfect hands (soap and water, rinse with potable water then rinse with 70% alcohol) or gloves (dipped in iodophore solution or other suitable disinfectant then rinsed with potable water) prior to shucking shellfish. Alternatively, use disposable gloves disinfected with 70% alcohol. A protective mail glove may be worn under the disposable glove to prevent accidental injury. Using a sterile shucking knife, open the shellfish through the bill, not hinge, and collect meats and liquor into a sterile container.

5.3.5. Weigh at least 200 g of shellfish and liquor into a tared blender jar and add an equal amount of 0.5% peptone water. Blend for 1 - 2 minutes. Blended homogenate represents a 1 in 2 dilution.

5.3.6. To obtain a 1 in 10 dilution, add 20 g of the homogenate to 80 g of peptone water and shake. Shake dilutions 25 times through a 1-foot (30 cm) arc in approximately 7 seconds.

5.3.7. Prepare succeeding decimal dilutions as required using a separate sterile pipette for making each transfer.

5.3.8. Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

5.3.9. Immediately (i.e., within 2 minutes after blending) prepare the dilutions from the ground sample and then proceed to inoculate into tubes. Inoculate each of separate sets of five tubes of LST broth with each dilution to be tested, according to the scheme in (Table II) as follows:

Inoculate shellfish samples into LST: 10 mL of a 1 in 10 dilution into each of 5 tubes of double strength LST, 1 mL of 1 in 10 dilution into each of 5 tubes of single strength LST, and 1 mL of 1 in 100 dilution to each of 5 tubes of single strength LST.

5.3.10. Follow incubation of LST and confirmation steps for coliforms, faecal coliforms and *E. coli* as required, and record results as MPN per 100 g of shellfish.

5.4. Preparation of Sample, Initial Set-up and Reporting - Water

5.4.1. Inoculate each of separate sets of five tubes of LST broth with each dilution to be tested, according to the scheme in (Table II), as follows.

Inoculate each of the five tubes of 10 mL double strength LST broth (first row) with 10 mL of the undiluted water sample. Inoculate each of the five tubes of 10 mL single strength LST broth (second row) with 1 mL undiluted water. Inoculate each of the five tubes of 10 mL single strength LST broth (third row) with 0.1 mL of undiluted water.

5.4.2. Follow incubation of LST and confirmation steps for coliforms, faecal coliforms and *E. coli* as required, and record results as MPN per 100 mL of water

5.5. Preparation of Sample, Initial Set-up and Reporting - All other commodities

5.5.1. To ensure a truly representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit. To reduce the workload, the

analytical units may be combined for analysis. It is recommended that a composite contain not more than 500 g.

5.5.2. Prepare a 1 in 10 dilution of the food by aseptically blending 11 (10) g or mL (the analytical unit) into 99 (90) mL of the required diluent, as indicated in Tables I and II. If five sub-samples are composited for analysis, aseptically blend 50 g or mL into 450 mL of the required diluent.

For fish products an alternative method may be used. Weigh 100 g fish products and add 300 mL of 0.1% peptone water. Blend for 2 minutes. Blended homogenate represents a 1 in 4 dilution. Weigh 40 g of homogenate into 60 mL of 0.1 % peptone to obtain a 1 in 10 dilution. Pipette into LST as in 5.3.9 and express results as MPN/100g.

5.5.3. With products that require blending, blend or stomach for the minimum time required to produce a homogeneous suspension and to avoid overheating, blending time should not exceed 2.5 min. When blending foods that tend to foam, use blender at low speed and remove aliquot from below liquid/foam interface.

5.5.4. Check pH of the food suspension. If the pH is outside the range of 5.5-7.5, adjust pH to 7.0 with sterile 1N NaOH or 1N HCl.

5.5.5. Allow the food homogenate (1 in 10 dilution) of dry foods to stand at room temperature for 15 min. In all other instances, continue the analysis without this delay.

5.5.6. Prepare succeeding decimal dilutions as required using a separate sterile pipette for making each transfer. Shake dilutions 25 times through a 1-foot (30 cm) arc in approximately 7 seconds.

5.5.7. Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

5.5.8. Inoculate each of separate sets of five tubes of LST broth with each dilution to be tested, according to the scheme in (Table II) as follows.

5.5.9. Inoculate each of the five tubes of 10 mL single strength LST broth (first row) with 1 mL of the10^{-1} dilution. Inoculate each of the five tubes of succeeding rows of single strength LST with 1 mL additional dilutions.

5.5.10. Follow incubation of LST and confirmation steps for coliforms, faecal coliforms and *E. coli* as required. Compute MPN per g (mL) of food (per 100 g of shellfish or fish products or per 100mL of water) convert the number of gas-positive tubes to MPN values.

5.6. Incubation of LST

5.6.1. In order to verify growth conditions in the elevated temperature water baths, inoculate one LST broth tube with the MPN broths positive control and one LST broth tube with the MPN negative control, for each bath used. Transfer into all media used at different stages of the procedure. Set up an uninoculated tube of medium corresponding to each step in the procedure as a media control.

5.6.2. Mix inoculum and medium by gently shaking or rotating the tubes, but avoid entrapping air in the gas vials.