result of contamination from rodents and birds. Filter-feeding shellfish harvested from polluted waters and frozen precooked prawns have been identified as higher risk products.

Since birds, rodents, insects, infected food handlers or infected foods can all contaminate foods directly or indirectly, potential food vehicles for salmonella are numerous. Contaminated cocoa beans which had been processed into chocolate were responsible for outbreaks of *S*. Eastbourne in the United States and Canada, of *S*. Napoli and *S*. Montevideo in England, and *S*. Typhimurium in Scandinavia. Although the production of chocolate involves a heating stage, this was insufficient to kill all the salmonellas present, possibly as a result of a protective effect from the cocoa butter.

Desiccated coconut is used in a range of confectionary products and was identified as a hazard following cases of typhoid and salmonellosis in Australia. In 1959/60, a survey of desiccated coconut imports into the UK from Sri Lanka revealed that 9% of samples contained *Salmonella*. In response, the introduction and enforcement of regulations in Sri Lanka to improve production hygiene have now reduced the contamination rate dramatically.

Other plant products such as salad vegetables have been associated with occasional outbreaks of typhoid and salmonellosis. Use of polluted irrigation water or human and animal manure as fertilizer can be important contributory factors in such cases.

7.13 *SHIGELLA*

7.13.1 Introduction

The genus *Shigella* was discovered as the cause of bacillary dysentery by the Japanese microbiologist Kiyoshi Shiga in 1898. It consists of four species *Sh. dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei*, all of which are regarded as human pathogens though they differ in the severity of the illness they cause. *Sh. dysenteriae* has been responsible for epidemics of severe bacillary dysentery in tropical countries but is now rarely encountered in Europe and North America where *Sh. sonnei* is more common. *Sh. sonnei* causes the mildest illness, while that caused by *Sh. boydii* and *Sh. flexneri* is of intermediate severity.

Although *Shigella* is relatively inactive biochemically when compared with *Escherichia* species, studies of DNA relatedness have demonstrated that they do in fact belong to the same genus. The separate genera are retained however, because, unlike *Escherichia*, most strains of *Shigella* are pathogenic and a redesignation might cause confusion with potentially serious consequences.

Laboratory reports of *Sh. sonnei* infections in England and Wales rose to 9830 in 1991 compared to 2319 and 2228 in the previous two years. In the United States annual reports over recent years have ranged between 300 000 and 450 000. Shigellas are spread primarily person-to-person by the faecal–oral route although foodborne outbreaks have been recorded. Some experts consider that the problem of foodborne shigellosis is greatly underestimated.

7.13.2 The Organism and its Characteristics

Shigellas 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 usually no gas from glucose and, with the exception of some strains of *S. sonnei*, are unable to ferment lactose; a feature they share with most salmonellas.

Shigellas are generally regarded as rather fragile organisms which do not survive well outside their natural habitat which is the gut of humans and other primates. They have not attracted the attention that other foodborne enteric pathogens have, but such evidence as is available suggests that their survival characteristics are in fact similar to other members of the Enterobacteriaceae. They are typical mesophiles with a growth temperature range between 10–45 °C and a heat sensitivity comparable to other members of the family. They grow best in the pH range 6–8 and do not survive well below pH 4.5. A number of studies have reported extended survival times in foods such as flour, pasteurized milk, eggs and shellfish.

The species are distinguished on the basis of biochemical tests and both serotyping and phage typing schemes are available for further subdivision of species.

7.13.3 Pathogenesis and Clinical Features

Shigellas cause bacillary dysentery in humans and other higher primates. Studies with human volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. The incubation period can vary between 7 h and 7 days although foodborne outbreaks are commonly characterized by shorter incubation periods of up to 36 h. Symptoms are of abdominal pain, vomiting and fever accompanying a diarrhoea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus, in the cases of *Sh. dysenteriae*, *Sh. flexneri* and *Sh. boydii*, to a watery diarrhoea with *Sh. sonnei*. Illness lasts from 3 days up to 14 days in some cases and a carrier state may

develop which can persist for several months. Milder forms of the illness are self-limiting and require no treatment but *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy.

Shigellosis is an invasive infection where the organism's invasive property is encoded on a large plasmid. Other details of the pathogenesis of the infection are described in Chapter 6 (Section 6.7).

7.13.4 Isolation and Identification

Lack of interest in *Shigella* as a foodborne pathogen has meant that laboratory protocols for its isolation and identification from foods are relatively underdeveloped. A pre-enrichment procedure has been described based on resuscitation on a non-selective agar before overlaying with selective media. Selective enrichment in both Gram-negative broth and selenite broth has been recommended. Selective plating media used are generally those employed for enumerating the Enterobacteriaceae or *Salmonella* although neither are entirely satisfactory.

Rapid techniques for identification based on immunoassays which detect the virulence marker antigen, and on the polymerase chain reaction to detect the virulence plasmid by DNA/DNA hybridization have also been applied.

7.13.5 Association with Foods

Foodborne cases of shigellosis are regarded as uncommon though some consider the problem to be greatly underestimated. The limited range of hosts for the organism certainly suggests that it is relatively insignificant as a foodborne problem when compared with say *Salmonella*.

In foodborne cases, the source of the organism is normally a human carrier involved in preparation of the food. In areas where sewage disposal is inadequate the organism could be transferred from human faeces by flies. Contamination during primary production of a crop was responsible for an extensive outbreak of *Sh. sonnei* which affected several European countries in 1994 and was associated with imported iceberg lettuce.

Uncooked foods which may have received extensive handling such as prawn cocktail or tuna salad have been implicated in a number of outbreaks. In one, which occurred in Cambridgeshire, England, in 1992, 107 out of 200 guests at a buffet meal developed diarrhoea and *Sh. sonnei* was isolated from 81 of 93 faecal samples taken. The organism was also isolated from two of the catering staff. Investigation revealed a strong association between illness and consumption of two prawn dishes for which both infected caterers had been involved in the preparation.

7.14 *STAPHYLOCOCCUS AUREUS*

7.14.1 Introduction

The staphylococci were first described by the Scottish surgeon, Sir Alexander Ogston as the cause of a number of pyogenic (pus forming) infections in humans. In 1882, he gave them the name staphylococcus (Greek: *staphyle*, bunch of grapes; *coccus*, a grain or berry), after their appearance under the microscope.

The first description of food poisoning caused by staphylococci is thought to be that of Vaughan and Sternberg who investigated a large outbreak of illness in Michigan believed to have been caused by cheese contaminated with staphylococci. Clear association of the organisms with foodborne illness had to wait until Barber (1914) demonstrated that staphylococci were able to cause poisoning by consuming milk from a cow with staphylococcal mastitis. In 1930, Dack showed that staphylococcal food poisoning was caused by a filterable enterotoxin.

There are currently 27 species and 7 subspecies of the genus *Staphylococcus*; enterotoxin production is principally asssociated with the species *Staph. aureus*, although it has also been reported in others including *Staph. intermedius* and *Staph. hyicus*.

As a relatively mild, short-lived type of illness, staphylococcal food poisoning is perhaps more likely to be under-reported than others. Most reported cases are associated with outbreaks and only a few sporadic cases are detected. In the United States between 1983 and 1987, staphylococci accounted for 7.8% (47) of the 600 bacterial food poisoning outbreaks that were recorded. Equivalent figures for England and Wales over the same period were 1.9% (54) out of a total of 2815 outbreaks. Outbreaks of staphylococcal food poisoning in the UK peaked during the 1950s at 150 outbreaks per year but have since declined to an annual level of 5–10 outbreaks in the period 1990 to 1996 and an average of one per year in the period 2000 to 2005.

7.14.2 The Organism and its Characteristics

Staphylococcus aureus is a Gram-positive coccus forming spherical to ovoid cells about 1 μm in diameter. Cell division occurs in more than one plane so that cells form irregular clumps resembling bunches of grapes (Figure 7.10).

Staphylococci are catalase-positive, oxidase-negative, facultative anaerobes. Their ability to ferment glucose can be used to distinguish them from the strictly respiratory genus *Micrococcus*, although there are species in both genera where this distinction is not clear cut due to low acid production by some staphylococci and production of small amounts

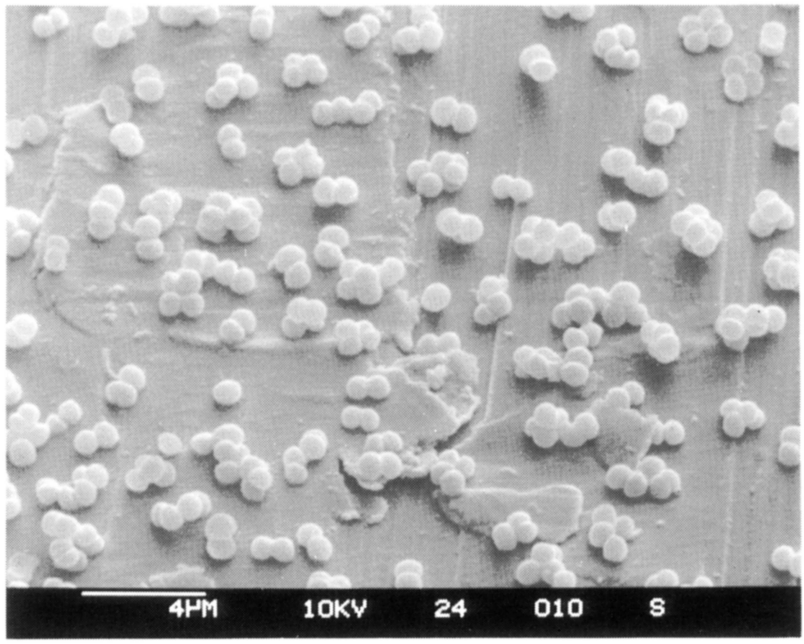

Figure 7.10 Staphylococcus aureus *attached to stainless steel.* (Photo M.Lo)

Table 7.8 *Factors permitting growth and enterotoxin production by* Staphylococcus aureus

	Growth		*Enterotoxin Production*	
Factor	*Optimum*	*Range*	*Optimum*	*Range*
Temperature,°C	35–37	7–48	35–40	10–45
pH	6.0–7.0	4.0–9.8	Ent. A. 5.3–6.8 others 6–7	4.8–9.0
NaCl	0.5–4.0%	0–20%	0.5%	0–20%
Water activity	0.98–>0.99	0.83–>0.99	>0.99	0.86–>0.99
Atmosphere	Aerobic	Aerobic-Anaerobic	5–20% DO_2	Aerobic-Anaerobic
E_h	>+200mV	<–200 to >+200mV	>+200mV	?

of acid under anaerobic conditions by some micrococci. Enterotoxin production is adversely affected by anaerobic conditions far more than growth.

Staphylococcus aureus is a typical mesophile with a growth temperature range between 7 and 48 °C and an optimum at 37 °C under otherwise optimal conditions. The range of temperature over which enterotoxin is produced is narrower by a few degrees and has an optimum at 35–40 °C (Table 7.8). The organism has unexceptional heat resistance with a D_{62} of 20–65 s and a D_{72} of 4.1 s when measured in milk using

log-phase cultures. Heat resistance has been shown to vary considerably though, and D values were found to increase three-fold when stationary-phase cultures were tested.

Growth occurs optimally at pH values of 6–7, with minimum and maximum limits of 4.0 and 9.8–10.0 respectively. The pH range over which enterotoxin production occurs is narrower with little toxin production below pH 6.0 but, as with growth, precise values will vary with the exact nature of the medium.

A characteristic of *Staph. aureus* which is a particularly important consideration in some foods is its tolerance of salt and reduced a_w. It grows readily in media containing 5–7% NaCl and some strains are capable of growth in up to 20% NaCl. It will grow down to an a_w of 0.83 where it has a generation time of 300 min. Once again the range over which enterotoxin production occurs is more limited with a minimum a_w recorded of 0.86.

The principal habitat of the staphylococci is the skin, skin glands and the mucous membranes of warm blooded animals. Several species are associated with particular hosts, for example *Staph. hyicus* with pigs, and *Staph. gallinarum* with chickens. *Staph. aureus* is more widespread 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.

Though normally a harmless parasite of human body surfaces where it plays a useful role metabolizing skin products and possibly preventing skin colonization by pathogens, *Staph. aureus* can cause minor skin abscesses such as boils and, more seriously, as an opportunistic pathogen when the skin barrier is breached or host resistance is low.

7.14.3 Pathogenesis and Clinical Features

Food poisoning by *Staph. 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 days. In severe cases dehydration, marked pallor and collapse may require treatment by intravenous infusion.

The short incubation period is characteristic of an intoxication where illness is the result of ingestion of a pre-formed toxin in the food. *Staph. aureus* produces at least 11 enterotoxins designated SEA to SEJ. To add a touch of Byzantine complexity and confuse the unwary there is no SEF and there are three variants of SEC. 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 less than 1 μg of pure toxin has been required to elicit symptoms. The toxins are small (M_r 26–30 kDa) single-chain polypeptides which share considerable amino acid homology. With the exception of SEI each contains 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. Such procedures would of course eliminate viable *Staph. aureus* from a food so it is possible for someone to become ill from eating a food which contains no viable *Staph. aureus*.

Though frequently described as enterotoxins the *Staph. aureus* toxins are strictly neurotoxins. They elicit the emetic response by acting on receptors in the gut, which stimulate the vomiting centre in the brain *via* the vagus and sympathetic nerves. If these nerves are severed then vomiting does not occur. It is not known how the toxin induces diarrhoea but it has been shown not to stimulate adenylate cyclase activity.

The *Staph. aureus* enterotoxins are now also known to be superantigens, molecules that are able to stimulate a much higher percentage of T cells than conventional antigens. What role this may play in gastrointestinal illness, if any, is not known.

7.14.4 Isolation and Identification

The most successful and widely used selective plating medium for *Staph. aureus* is the one devised by Baird-Parker in the early 1960s. It combines the virtues of a high degree of selectivity, a characteristic diagnostic reaction, and the ability to recover stressed cells. Lithium chloride and tellurite act as selective agents while egg yolk and pyruvate assist in the recovery of damaged cells. Reduction of the tellurite by *Staph. aureus* gives characteristic shiny, jet-black colonies which are surrounded by a zone of clearing, resulting from hydrolysis of the egg-yolk protein lipovitellenin. Colonies also often have an inner white margin caused by precipitation of fatty acid.

Colonial appearance on Baird-Parker (B-P) agar gives presumptive identification of *Staph. aureus* which is often confirmed by tests for the production of coagulase and thermostable nuclease.

Coagulase is an extracellular substance which coagulates human or animal blood plasma in the absence of calcium. It is not specific to *Staph. aureus* but is also produced by *Staph. intermedius* and *Staph. hyicus*. *Staph. intermedius* is unable to reduce tellurite and therefore produces white colonies on B-P agar, but *Staph. hyicus*, which is found on the skin

of pigs and poultry, requires a series of further biochemical tests to distinguish it reliably from *Staph. aureus*.

The presence of coagulase can be demonstrated using EDTA-treated rabbit plasma in the tube coagulase test. More rapid test kits are available, based on the detection of bound coagulase (also known as clumping factor) and/or protein A, which reacts with the Fc part of IgG molecules. Detection is by agglutination of erythrocytes or latex particles coated with fibrinogen or plasma and colonies from selective media can be tested directly, without any intermediate sub-culturing. Coagulase production can also be detected directly in an egg yolk-free modification of B-P agar containing pig or rabbit plasma.

Detection of thermostable nuclease uses toluidine blue/DNA agar either with a boiled culture supernatant or as an overlay on heat-treated colonies on B-P agar.

Four biotypes of *Staph. aureus* are recognized but the use of biotyping is limited since nearly all of the strains isolated from human sources belong to biotype A. Phage typing schemes are used with *Staph. aureus*; most food poisoning strains belonging to serogroup III.

Since the enterotoxins will survive heat processes that eliminate the producing organism, toxin detection in a food is a more reliable indication of hazard than viable counting procedures. A number of immunoassay techniques for staphylococcal enterotoxins are available. Early immunoprecipitation techniques such as the microslide gel diffusion test are less sensitive and require lengthy extraction and concentration procedures to isolate sufficient enterotoxin for detection. ELISA techniques which will detect 0.1–1.0 ng toxin g^{-1} food and reverse passive latex agglutination tests with a sensitivity of 0.5 ng ml^{-1} are now available and more widely used.

7.14.5 Association with Foods

The presence of small numbers of *Staph. aureus* on foods is not uncommon. It will occur naturally in poultry and other raw meats as a frequent component of the skin microflora. Similarly, it can be isolated from raw milk where levels may sometimes be elevated as a result of *Staph. aureus* mastitis in the producing herd. As a poor competitor, it normally poses no problem in these situations since it does not grow and is eliminated by cooking or pasteurization. There have however been outbreaks caused by milk products such as dried milk and chocolate milk where growth and enterotoxin production occurred in the raw milk and the enterotoxin, but not the organism, survived subsequent pasteurization. A good example of this is a large outbreak that occurred in Japan in 2000, affecting more than 13,000 people. A power cut during production of dried skimmed milk led to delays in processing that allowed *Staph.*

aureus to multiply and produce enterotoxin. The contaminated powder was then used in a number of dairy products. Though not in itself a health threat, the presence of *Staph. aureus* on raw meats does pose the risk of cross-contamination of processed food.

Contamination by food handlers is also probably a frequent occurrence in view of the high rate of human carriage. Colonization of the nose and throat with the organism will automatically imply its presence on the skin and food may also be contaminated from infected skin lesions or by coughing and sneezing. Since large numbers, typically $> 10^6\,g^{-1}$, are required for the production of enough toxin to cause illness, contamination is necessary but is not alone sufficient for an outbreak to occur. In particular, temperature and time conditions must also be provided that allow the organism to grow.

Studies in the United States and the UK have found that poultry products and cold, cooked meats are the most common vehicles. Salted meats such as ham and corned beef are particularly vulnerable since the *Staph. aureus* is unaffected by levels of salt that will inhibit a large proportion of the competitive flora. Buffet meals where such meats are served are a common scenario for outbreaks as the food is necessarily prepared some time in advance and too often stored at ambient temperature or inadequately chilled.

Canned foods also offer *Staph. aureus* a congenial, competitor-free environment and post-process leakage contamination of cans has been an occasional cause of outbreaks.

Other outbreaks have been caused by hard cheeses, cold sweets, custards and cream-filled bakery products. In Japan, rice balls that are moulded by hand are the commonest vehicle while in Hungary, it is ice cream.

7.15 *VIBRIO*

7.15.1 Introduction

Historically, cholera has been one of the diseases most feared by mankind. It is endemic to the Indian subcontinent where it is estimated to have killed more than 20 million people this century. During the 19th century there were a number of pandemics of 'Asiatic cholera' spreading from the Indian subcontinent throughout Europe and the Americas. It spread inexorably across Europe at a rate of about eight kilometres a day reaching England in 1831, where it thrived in the appalling overcrowded, insanitary conditions of the burgeoning towns and cities. The approach of a second outbreak in 1848 prompted Parliament to establish the Central Board of Health which began the long task of improving sewerage and water supply systems. Similar apprehension of an

approaching cholera outbreak in 1866 inspired the foundation of a similar Board in New York in the United States.

Pacini (1854) is credited with the first description of the etiological agent of cholera when he observed large numbers of curved bacilli in clinical specimens from cholera patients in Florence. His findings were not however generally accepted because of the widespread occurrence of similar but harmless vibrios in the environment. It was Robert Koch who firmly established the causal link between *Vibrio cholerae* and cholera when working in Egypt in 1886.

Koch isolated what is now known as the classical *V. cholerae* biotype which was responsible for most outbreaks of cholera until 1961. The *El Tor* biotype, first isolated in 1906 by Gotschlich from pilgrims bound for Mecca at the El Tor quarantine station in Sinai, Egypt, is responsible for the current (7th) pandemic. This started in Celebes (Sulawesi) in Indonesia in 1961, reached Africa in 1970 and the Americas in 1991. Of the 594 694 cases reported to the WHO in 1991, 391 220 were in South and Central America.

It was recognized in the 1930s that both biotypes are agglutinated by a single antiserum designated O1. Other strains of *V. cholerae* do not react with this antiserum and are termed non-agglutinable, or more correctly non-O1 strains, though some do produce cholera toxin. In 1992 a new serotype, O139, was associated with epidemic cholera in India and Bangladesh and has also been isolated from cholera patients in Thailand.

A number of other species of *Vibrio* have been recognized as pathogens causing wound and ear infections, septicaemia as well as gastrointestinal upsets (Table 7.9). In particular, *V. parahaemolyticus*, which was first shown to be an enteropathogen in 1951, is responsible for 50–70% of outbreaks of foodborne gastroenteritis in Japan. *V. fluvialis* has been isolated from sporadic cases of diarrhoea in some countries, particularly those with warm climates, although its exact role is uncertain since other enteropathogens were often present in the stool samples. *V. mimicus*,

Table 7.9 Vibrio *species associated with human diseases*

Species	*Disease*
V. cholerae , O1	Cholera, wound infection
V. cholerae, non-O1	Diarrhoea, gastroenteritis, wound infection, secondary septicaemia
V. mimicus	Diarrhoea, gastroenteritis, wound infection
V. parahaemolyticus	Gastroenteritis, wound infection, otitis media
V. fluvialis	Diarrhoea
V. furnissii	Diarrhoea
V. hollisae	Diarrhoea
V. vulnificus	Wound infection, primary septicaemia, secondary septicaemia
V. alginolyticus	Wound infection, otitis media
V. damsela	Wound infection

which produces diarrhoea, is distinguishable from *V. cholerae* only by its ability to produce acid from sucrose and acetoin from glucose. *V. vulnificus* does not usually cause diarrhoea but severe extra-intestinal infections such as a life-threatening septicaemia. Patients normally have some underlying disease and have eaten seafood, particularly oysters about a week before the onset of illness.

7.15.2 The Organisms and their Characteristics

Vibrios are Gram-negative pleomorphic (curved or straight), short rods which are motile with (normally) sheathed, polar flagella. Catalase and oxidase-positive cells are facultatively anaerobic and capable of both fermentative and respiratory metabolism. Sodium chloride stimulates the growth of all species and is an obligate requirement for some. The optimum level for the growth of clinically important species is 1–3%. *V. parahaemolyticus* grows optimally at 3% NaCl but will grow at levels between 0.5% and 8%. The minimum a_w for growth of *V. parahaemolyticus* varies between 0.937 and 0.986 depending on the solute used.

Growth of enteropathogenic vibrios occurs optimally at around 37 °C and has been demonstrated over the range 5 – 43 °C, although ≈10 °C is regarded as a more usual minimum in natural environments. When conditions are favourable, vibrios can grow extremely rapidly; generation times of as little as 11 min and 9 min have been recorded for *V. parahaemolyticus* and the non-pathogenic marine vibrio, *V. natrigens* respectively.

V. parahaemolyticus is generally less robust at extremes of temperatures than *V. cholerae*. Numbers decline slowly at chill temperatures below its growth minimum and under frozen conditions a 2-log reduction has been observed after 8 days at −18 °C. The D_{49} for *V. parahaemolyticus* in clam slurry is 0.7 min compared with a D_{49} for *V. cholerae* of 8.15 min measured in crab slurry. Other studies have recorded higher D values for *V. parahaemolyticus*, for instance 5 min at 60 °C produced only 4–5 log reductions in peptone/3% NaCl. Pre-growth of the organism in the presence of salt is known to increase heat resistance.

V. parahaemolyticus and other vibrios will grow best at pH values slightly above neutrality (7.5–8.5) and this ability of vibrios to grow in alkaline conditions up to a pH of 11.0 is exploited in procedures for their isolation. Vibrios are generally viewed as acid sensitive although growth of *V. parahaemolyticus* has been demonstrated down to pH 4.5–5.0.

The natural habitat of vibrios is the marine and estuarine environment. *V. cholerae* can be isolated from temperate, sub-tropical, and tropical waters throughout the world, but seem to disappear from

temperate waters during the colder months. Long-term survival may be enhanced by attachment to the surfaces of plants and marine animals and a viable but non-culturable form has also been described where the organism cannot be isolated from the environment using cultural techniques even though it is still present in an infective form.

V. parahaemolyticus is primarily associated with coastal inshore waters rather than the open sea. It cannot be isolated when the water temperature is below 15 °C and cannot survive pressures encountered in deeper waters. The survival of the organism through winter months when water temperatures drop below 15 °C has been attributed to its persistence in sediments from where it may be recovered even when water temperatures are below 10 °C.

Most environmental isolates of both *V. cholerae* and *V. parahaemolyticus* are non-pathogenic. The majority of the *V. cholerae* are non-O1 serotypes and even those that are O1 tend to be non-toxigenic. Similarly 99% of environmental strains of *V. parahaemolyticus* are non-pathogenic.

7.15.3 Pathogenesis and Clinical Features

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life-threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food or buffer, of the order of 10^{10} cells, but is considerably reduced if consumed with food which protects the bacteria from stomach acidity. Studies conducted in Bangladesh indicate that 10^3–10^4 cells may be a more typical infectious dose. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera.

Cholera is a non-invasive infection where the organism colonizes the intestinal lumen and produces a potent enterotoxin. Details of the cholera toxin and its mode of action are given in Chapter 6 (Section 6.7). In severe cases, the hypersecretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhoea containing flakes of mucus, described as rice water stools. The diarrhoea, which can be up to 20 l day^{-1} and contains up to 10^8 vibrios ml^{-1}, is accompanied by vomiting, but without any nausea or fever.

Unless the massive losses of fluid and electrolyte are replaced, there is a fall in blood volume and pressure, an increase in blood viscosity, renal failure, and circulatory collapse. In fatal cases death occurs within a few days. In untreated outbreaks the death rate is about 30–50% but can be reduced to less than 1% with prompt treatment by intravenous or oral rehydration using an electrolyte/glucose solution.

The reported incubation period for *V. parahaemolyticus* food poisoning varies from 2 h to 4 days though it is usually 9–25 h. Illness persists for up to 8 days and is characterized by a profuse watery diarrhoea free from blood or mucus, abdominal pain, vomiting and fever. *V. parahaemolyticus* is more enteroinvasive than *V. cholerae*, and penetrates the intestinal epithelium to reach the *lamina propria*. A dysenteric syndrome has also been reported from a number of countries including Japan.

Pathogenicity of *V. parahaemolyticus* strains is strongly linked to their ability to produce a 22 kDa, thermostable, extracellular haemolysin. When tested on a medium known as Wagatsuma's agar, the haemolysin can lyse fresh human or rabbit blood cells but not those of horse blood, a phenomenon known as the Kanagawa reaction. The haemolysin has also been shown to have enterotoxic, cytotoxic and cardiotoxic activity.

Most (96.5%) strains from patients with *V. parahaemolyticus* food poisoning produce the haemolysin and are designated Kanagawa positive (Ka+) while 99% of environmental isolates are Ka–. Volunteer feeding studies have found that ingestion of 10^7–10^{10} Ka– cells has no effect whereas 10^5–10^7 Ka+ cells produce illness. A number of other virulence factors have been described but have been less intensively studied.

V. vulnificus is a highly invasive organism that causes a primary septicaemia with a high fatality rate ($\approx$50%). Most of the cases of foodborne transmission identified occurred in people with pre-existing liver disease, diabetes or alcoholism. Otherwise healthy individuals are rarely affected and, when they are, illness is usually confined to a gastroenteritis. In foodborne cases, the symptoms of malaise followed by fever, chills and prostration appear 16–48 h after consumption of the contaminated food, usually seafoods, particularly oysters. Unlike other vibrio infections, *V. vulnificus* infections require treatment with antibiotics such as tetracycline.

7.15.4 Isolation and Identification

The enrichment media used for vibrios exploit their greater tolerance for alkaline conditions. In alkaline peptone water (pH 8.6–9.0) the incubation period must be limited to 8 h to prevent overgrowth of the vibrios by other organisms. Tellurite/bile salt broth (pH 9.0–9.2) is a more selective enrichment medium and can be incubated overnight.

The most commonly used selective and differential agar used for vibrios is thiosulfate/citrate/bile salt/sucrose agar (TCBS). The medium was originally designed for the isolation of *V. parahaemolyticus* but other enteropathogenic vibrios grow well on it, with the exception of *V. hollisae*. *V. parahaemolyticus*, *V. mimicus*, and *V. vulnificus* can be distinguished from *V. cholerae* on TCBS by their inability to ferment sucrose which results in the production of green colonies. *V. cholerae*

produces yellow colonies. Individual species can then be differentiated on the basis of further biochemical tests.

V. cholerae is divided into the serogroups O1 and non-O1. O1 strains can be further classified into the classical (non-haemolytic) or El Tor (haemolytic) biotypes each of which can be subdivided by serotyping into one of three groups: Ogawa, Inaba or Hikojima. These can be further subdivided by phage typing although with the advent of molecular typing techniques this is less commonly used. Clinical strains of *V. parahaemolyticus* can be serotyped for epidemiological purposes using a scheme based on 11 thermostable O antigens and 65 thermolabile K (capsular) antigens.

7.15.5 Association with Foods

Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle. Consequently a large number of different foods have been implicated in outbreaks, particularly products such as washed fruits and vegetables which are consumed without cooking. Foods coming from a contaminated environment may also carry the organism, for example seafoods and frog's legs. In the current pandemic in South and Central America, an uncooked fish marinade, in lime or lemon juice, *ceviche* has been associated with some cases.

V. parahaemolyticus food poisoning is invariably associated with fish and shellfish. Occasional outbreaks have been reported in the United States and Europe, but in Japan it is the commonest cause of food poisoning. This has been linked with the national culinary habit of consuming raw or partially cooked fish, although illness can also result from cross-contamination of cooked products in the kitchen. Though the organism is only likely to be part of the natural flora of fish caught in coastal waters during the warmer months, it can readily spread to deep-water species through contact in the fish market and it will multiply rapidly if the product is inadequately chilled.

The risk of *V. vulnificus* infection associated with raw oysters is most effectively controlled by cooking the product before consumption. Susceptible individuals, such as those described previously, and the immunosuppressed should avoid consumption of uncooked shellfish.

7.16 *YERSINIA ENTEROCOLITICA*

7.16.1 Introduction

Yersinia enterocolitica is one of three species of the genus *Yersinia* recognized as human pathogens; *Y. enterocolitica* causes predominantly

a gastroenteritis, while Y. *pseudotuberculosis* is associated mainly with mesenteric adenitis. In terms of their social impact, both pale into insignificance when compared to *Yersinia pestis*, responsible for the bubonic plague which killed an estimated 25% of the European population in the 14th Century.

The genus *Yersinia* is named after the French bacteriologist Alexandre Yersin who, in 1894, first described the organism responsible for the bubonic plague. It was created to accommodate former members of the genus *Pasteurella* that were clearly members of the Enterobacteriaceae. In 1964, comparison of *Bacterium enterocoliticum* with a number of closely related isolates, identified by other workers as *Pasteurella* spp., led Frederiksen to propose the creation of the new species *Yersinia enterocolitica*. Further definition within the genus has occurred with the creation of seven new species from non-pathogenic strains previously described as '*Yersinia enterocolitica-like*'. Those most commonly isolated from foods, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii* and *Y. bercovierii* can be readily distinguished from *Y. enterocolitica* on the basis of a few biochemical tests.

The importance of *Y. enterocolitica* as a cause of foodborne illness varies between countries. In England and Wales, laboratory reports of *Y. enterocolitica* infections, mostly sporadic cases, increased from 45 in 1980 to 571 in 1989 when it outnumbered cases of both *Staph. aureus* and *Bacillus* food poisoning. This represented a peak in reports which then declined to an average of 25 p.a. in the period 2000 to 2005.

Yersiniosis is most common in the cooler climates of northern Europe, particularly in Belgium, and in North America where a number of large outbreaks have been reported. It also displays a different seasonal variation from most other foodborne pathogens with a peak in reported cases occurring in the autumn and winter.

7.16.2 The Organism and its Characteristics

Yersinia enterocolitica is a member of the Enterobacteriaceae; an asporogenous, short (0.5–1.0 by 1–2 μm) Gram-negative rod which is facultatively anaerobic, catalase-positive and oxidase-negative. It can grow over a wide range of temperature, from $-1\,^{\circ}C$ to $+40\,^{\circ}C$, with an optimum around $29\,^{\circ}C$ and has a number of temperature-dependent phenotypic characteristics. For example, it is non-motile at $37\,^{\circ}C$, but motile with peritrichous flagella below $30\,^{\circ}C$. Like other psychrotrophs, though able to grow at chill temperatures, it does so slowly and at $3\,^{\circ}C$ has been found to take 4 days to increase by 2-log cycles in broth media.

It is heat sensitive but with considerable variation between strains; measured D values in whole milk at $62.8\,^{\circ}C$ have varied from 0.7–57.6 s.

Optimal growth occurs at a pH 7–8 with a minimum (in broth at 25 °C) varying between 5.1 and 4.1 depending on the acidulant used. As the temperature decreases so the minimum growth pH increases. Growth is possible in broth media containing 5% salt but not 7% salt at 3 °C or 25 °C.

Y. enterocolitica can be isolated from a range of environmental sources including soil, fresh water and the intestinal tract of many animals. Surveys have found the organism in numerous foods including milk and dairy products, meats, particularly pork, poultry, fish and shellfish, fruits and vegetables.

Most food isolates are however non-pathogenic and are known as environmental strains. The species can be subdivided by biotyping, serotyping and phage typing and pathogenicity appears to be associated only with certain types, each with a particular geographical distribution (Table 7.10). In Europe, Canada, Japan and South Africa human yersiniosis is most frequently caused by biotype 4, serotype O3 (4/O3) and, to a lesser extent in Europe and Japan, bio-serotype 2/O9. Strains of bio-serotype 4/O3 from Europe, Canada and South Africa can be distinguished by phage typing. In the United States bio-serotype 1/O8 most commonly causes human yersiniosis, although a wider range of bio-serotypes is encountered, e.g. 1/O13a; 1/O13b; 2/O5,27.

A number of techniques other than biotyping and serotyping have been described which claim to distinguish pathogenic from environmental strains of *Y. enterocolitica* relatively simply and are therefore more within the capacities of routine laboratories. These include the ability of pathogenic strains to autoagglutinate at 37 °C, their dependency on calcium for growth at 37 °C, and their uptake of Congo red dye, and they are usually associated with the presence of the 40–48 MDa virulence

Table 7.10 *Relationship between bio-sero-phage type of* Yersinia enterocolitica *host and geographical distribution*

Biotype	*Serotype*	*Phage type*	*Host*	*Syndrome*	*Country*
1	O8	X	Man	Gastroenteritis	USA, Canada
2	O9	X_3	Man	Gastroenteritis	Europe, Japan
			Pigs	Healthy	Europe, Japan
3	O1	II	Chinchillas	Systemic infection	Europe
4	O3	VIII	Man	Gastroenteritis	Europe, Japan
			Pigs	Healthy	Europe, Japan
		IXA	Man	Gastroenteritis	South Africa
			Pigs	Healthy	South Africa
		IXB	Man	Gastroenteritis	Canada
			Pigs	Healthy	Canada
5	O2	XI or II	Hares	Death	Europe
			Goats		

From S.J. Walker, PhD 1986

plasmid (see below). These tests are not completely reliable due to a number of problems such as the expression of plasmid-encoded phenotype in culture, occurrence of atypical strains and the possibility of plasmid loss during isolation. A test for pyrazinamidase activity which is not plasmid mediated may offer some advantages in this respect.

7.16.3 Pathogenesis and Clinical Features

Illness caused by *Y. enterocolitica* occurs most commonly in children under seven years old. It is a self-limiting enterocolitis with an incubation period of 1–11 days and lasting for between 5 and 14 days, although in some cases it may persist for considerably longer. Symptoms are predominantly abdominal pain and diarrhoea accompanied by a mild fever; vomiting is rare. Sometimes the pain resulting from acute terminal ileitis and mesenteric lymphadenitis (inflammation of the mesenteric lymph nodes) is confined to the lower right hand side of the body and prompts a mistaken diagnosis of appendicitis and subsequent surgery. A problem of post-infection complications such as arthritis and erythema nodosum (a raised, red skin lesion) can occur in adults, the latter particularly in women. This appears to be mainly associated with serotypes O3 and O9 and is therefore more common in Europe.

Ingested cells of pathogenic *Y. enterocolitica* which survive passage through the stomach acid adhere to the mucosal cells of the Peyer's patches (gut-associated lymphoid tissue). Adhesion is mediated through bacterial outer membrane proteins that are encoded for on a 40–48 MDa plasmid possessed by all pathogenic *Y. enterocolitica*. The plasmid is essential but not the sole prerequisite for virulence since cell invasion is controlled by chromosomal genes (see p.229). The adhered cell is taken up by the epithelial cell by endocytosis where it survives without significant multiplication and can exert cytotoxic activity. Released into the *lamina propria*, it invades phagocytic cells and multiplies extracellularly producing a local inflammatory response. Damage to the absorptive epithelial surface results in malabsorption and a consequent osmotic fluid loss characterized by diarrhoea. Other plasmid-encoded characteristics are thought to contribute to this process, such as the production of outer membrane proteins that confer resistance to phagocytosis, autoagglutination at 37 °C, and resistance to serum.

A heat-stable enterotoxin (9000–9700 Da) is produced by *Y. enterocolitica* but its role in pathogenesis, if any, is unclear. It bears some similarity to *E. coli* ST immunologically and in its ability to induce fluid accumulation in ligated ileal loops and to stimulate guanylate cyclase activity. Elaboration of enterotoxin in the gut is unlikely since production usually ceases at temperatures above 30 °C. Production in inoculated foods has been shown, but the observed incubation period is

inconsistent with a foodborne intoxication. Finally, the ability to produce the toxin is not confined to pathogenic *Y. enterocolitica*, but has also been demonstrated in numerous environmental strains and a number of other *Yersinia* species as well.

7.16.4 Isolation and Identification

A large number of procedures for the isolation and detection of *Y. enterocolitica* have been developed. Enrichment procedures usually exploit the psychrotrophic character of the organism by incubating at low temperature, but this has the disadvantage of being slow with the attendant possibility of overgrowth by other psychrotrophs present. Some workers have included selective agents in their enrichment media but some strains, such as serotype O8, are reported to be sensitive to selective agents. The most commonly used enrichment media are phosphate buffered saline (PBS) or tryptone soya broth (TSB) most usually incubated at 4 °C for 21 days. *Y. enterocolitica* and related species are more alkali resistant than many other bacteria so the pH of enrichment media is sometimes adjusted to 8.0–8.3 or cultures subjected to a short post-enrichment alkali treatment.

The best results for the selective isolation of *Y. enterocolitica* from foods and enrichment broths have been obtained with cefsulodin/ irgasan/novobiocin (CIN) agar. In addition to the antibiotics, the medium contains deoxycholate and crystal violet as selective agents and mannitol as a fermentable carbon source. After incubation at 28 °C for 24 h, typical colonies of *Y. enterocolitica* appear with a dark-red centre surrounded by a transparent border. Isolates can be confirmed and biotyped by biochemical tests.

In vitro tests to distinguish between environmental and pathogenic strains of *Y. enterocolitica* have been referred to above (Section 7.16.2). Techniques using gene probes to detect the virulence-associated plasmid by a colony hybridization test have also been used with some success and offer the possibility of detecting potentially pathogenic strains in foods without the need for lengthy enrichment procedures.

7.16.5 Association with Foods

Pigs are recognized as chronic carriers of those *Y. enterocolitica* serotypes most commonly involved in human infections (O3, O5, 27, O8, O9). The organism can be isolated most frequently from the tongue, tonsils and, in the gut, the caecum of otherwise apparently healthy animals. Despite this, pork has only occasionally been shown to be the vehicle for yersiniosis, although a case control study in Belgium, which has the highest incidence of yersiniosis, implicated a national prediliction

for eating raw pork. In 1988/9 an outbreak of yersiniosis in Atlanta involving 15 victims (14 children) was strongly associated with the household production of pork chitterlings.

A number of outbreaks of yersiniosis have been caused by contaminated milk including the largest hitherto recorded which occurred in 1982 in Tennessee, Arkansas and Mississippi in the United States. In this instance pigs were implicated as the original source of contamination, but not demonstrated to be carriers of the same O13 serotypes causing the infection. It was presumed that the organism was transferred from pigs, via mud, onto crates used to transport waste milk from the dairy to the pig farm. The crates were returned to the dairy and inadequately washed and sanitized before being used again to transport retail milk. As a consequence the outside of packs was contaminated with *Y. enterocolitica* which was transferred to the milk on opening and pouring. It was subsequently demonstrated that the organism involved could survive for at least 21 days on the outside of milk cartons held at 4 °C.

Contaminated water used in the production of beansprouts and in the packaging of tofu (soya bean curd) was responsible for two outbreaks in the United States in 1982.

A number of approaches to the control of yersiniosis have been proposed which are generally similar to those proposed for the control of other zoonotic infections such as salmonellosis. These include pathogen-free breeding and rearing of animals, a goal which may not be achievable in practice, and hygienic transport and slaughter practices. Work in Denmark on contamination of pork products with *Y. enterocolitica* has identified evisceration and incisions made during meat inspection as critical control points and has further shown that excision of the tongue and tonsils as a separate operation significantly reduces contamination of other internal organs.

7.17 SCOMBROTOXIC FISH POISONING

Scombrotoxic fish poisoning differs from those types of foodborne illness described above in that it is thought to be an example of where bacteria act as indirect agents of food poisoning by converting food components into harmful compounds. This view has however been questioned in recent years and discussion of scombrotoxicosis may belong more correctly in Chapter 8. Fish is almost always the food vehicle, particularly the so-called scombroid fish such as tuna, bonito and mackerel, but non-scombroid fish such as sardines, pilchards and herrings have also been implicated. In some cases canned fish has been responsible indicating that the toxic factor(s) is heat stable.

It is a chemical intoxication with a characteristically short incubation period of between 10 min and 2 h. Symptoms include a sharp, peppery

Table 7.11 *Guideline histamine levels in fish*

Histamine level Status (mg%)	*Status*
1	Freshly caught fish
<5	Normal and safe for consumption
5–20	Mishandled and possibly toxic
20–100	Unsatisfactory and probably toxic
>100	Toxic and unsafe for consumption

taste in the mouth, itching, dizziness, flushing of the face and neck, often followed by a severe headache, feverishness, diarrhoea, nausea and vomiting. A rash may develop on the face and neck and cardiac palpitations may occur.

The symptoms are those of histamine toxicity and can be alleviated with antihistamines. Histamine is produced by bacterial amino acid decarboxylases acting on histidine which occurs in high concentrations in the tissues of dark-fleshed fish. The bacteria themselves increase in numbers as a result of long storage at inappropriate temperatures and freshly caught fish have not been implicated in this type of poisoning.

When making judgements on the risk of scombrotoxic fish poisoning posed by particular products, regulatory authorities usually rely on a measure of the histamine content of the fish. In the United States, the level of histamine deemed hazardous in tuna is 50 mg%. Some guideline values published by the Health Protection Agency in the UK are presented as Table 7.11.

The problem is not as clear cut as it may at first seem, however. It has, for instance, not proved possible to reproduce the symptoms in volunteers fed histamine, and cases have also been reported where the fish contained low levels of histamine. Although histamine poisoning can occur when clearance of dietary histamine from the body is slowed by monoamine oxidase inhibitors, histamine is generally metabolized efficiently in the human gut and not absorbed *per se*. Among the explanations offered are that other biologically active amines are present in the fish which potentiate the toxicity of histamine or that algal toxins may be involved causing the release of endogenous histamine in the body.

7.18 CONCLUSION

In this chapter we have surveyed the main features of the foodborne bacterial pathogens recognized as being of current or emerging importance. As should be clear, the significance of individual organisms varies from country to country reflecting differences in both diet and culinary practices. It should also be remembered that the scene is likely to change

with time. The food microbiologist must be continually vigilant in anticipating the effect that changes in dietary preferences and social behaviour, and developments in crop and animal husbandry and food processing may have on bacterial hazards.

However, as noted in Chapter 6, not all the health risks posed by food are bacteriological. In the next Chapter we will consider some other microbiological hazards that are food associated.