1 Vibrio parahaemolyticus

1.1 Historical aspects

Fujino (1951) and Fujino et al. (1953) found a vibrio associated with a large outbreak of food poisoning occurring near Osaka, Japan. Later, Takikawa (1958) described another outbreak of gastroenteritis caused by an organism similar to the one isolated by Fujino et al. (1953). In 1960 an explosive epidemic of gastroenteritis occurred along the Pacific coast of Japan, and the Ministry of Health and Welfare established a committee to study this epidemic. Subsequently, a vibrio similar to the organism described by Fujino et al. and Takikawa was identified as the etiologic agent.

1.2 Characteristics

1.2.1 Classification and phenotypic characteristics

The organism was given different names, but the most widely accepted name, Vibrio parahaemolyticus, was suggested by Sakazaki et al. (1963). V. parahaemolyticus has a single polar, sheathed flagellum in broth culture, but a young culture on the surface of solid medium may have unsheathed, peritrichous flagella. The vibrio is halophilic, it grows in media containing 1–8 % sodium chloride, but not in media without salt. Many strains swarm on the surface of agar media. V. parahaemolyticus

1 deceased
Table 5.1 Phenotypic characteristics of *Vibrio* species associated with human disease

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth in peptone water with (% NaCl)</th>
<th>Susceptibility to O/129 (µg/ml)</th>
<th>Acid from</th>
<th>Gas from glucose</th>
<th>L-arabinose</th>
<th>Cellobiose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Salicin</th>
<th>Growth on TCBS agar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxidase</td>
<td>Nitratreduction</td>
<td>0</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>150</td>
<td>Flagellation</td>
<td>solid medium</td>
<td>Indole</td>
</tr>
<tr>
<td>V. cholerae</td>
<td>++</td>
<td>+</td>
<td>d</td>
<td>–</td>
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<td>–</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>M</td>
</tr>
<tr>
<td>V. mimicus</td>
<td>++</td>
<td>+</td>
<td>d</td>
<td>–</td>
<td>–</td>
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<td>S</td>
<td>S</td>
<td>M</td>
<td>+</td>
</tr>
<tr>
<td>V. para-haemolyticus</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>d</td>
<td>R</td>
<td>S</td>
<td>P</td>
</tr>
<tr>
<td>V. vulnificus</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
<td>S</td>
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<td>M</td>
<td>+</td>
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<tr>
<td>V. fluvialis</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>d</td>
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<td>–</td>
<td>R</td>
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<td>d</td>
</tr>
<tr>
<td>V. fimi hostile</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>d</td>
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<td>R</td>
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<tr>
<td>V. algolyticus</td>
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<td>+</td>
<td>–</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>S</td>
<td>P</td>
</tr>
<tr>
<td>V. mecht-honkovi</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>S</td>
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<td>d</td>
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<tr>
<td>V. hollisae</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>d</td>
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<td>–</td>
<td>R</td>
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</tr>
<tr>
<td>V. cinnammonis</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>+</td>
<td>–</td>
<td>R</td>
<td>S</td>
<td>M</td>
<td>+</td>
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<tr>
<td>V. carlchonae</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
<td>M</td>
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</tr>
<tr>
<td>V. damselfa</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
<td>M</td>
<td>+</td>
</tr>
</tbody>
</table>

+ 90–100 % strains positive; –, 0–10 % strains positive; d, 11–89 % strains positive; S, susceptible; R, resistant; M, monotrichous; P, peritrichous; Y, yellow; G, green.

*V. damselfa* is presently classified in the genus *Photobacterium.*
is susceptible to the vibrio-static agent O/129 (2,4-diamino-6,7-diisopropyl pteridine) at a concentration of 150 µg/ml but is resistant to 10 µg/ml. The phenotypic characteristics of *V. parahaemolyticus* are summarized in Table 5.1, together with those of other human pathogenic vibrios.

Although the flagellins of the polar and peritrichous flagella of the same strain differ in their immunological properties (Shinoda *et al.*, 1974), antigens made from intact flagellae of different strains of *V. parahaemolyticus* are serologically identical (Sakazaki *et al.*, 1968a; Terada, 1968). An antigenic scheme for *V. parahaemolyticus*, in which 11 O groups and 41 K antigens were recognized, was established by Sakazaki *et al.* (1968a); later, the number of K antigens was expanded to 70.

### 1.2.2 Ability to survive and grow in the environment

*V. parahaemolyticus* grows at temperatures between 10˚ and 44˚C, but fails to grow at 4˚C. Low temperatures not only arrest multiplication but also cause a rapid initial decrease in numbers of viable cells, although survival for several weeks occurs in refrigerated seafood. Under optimal conditions the generation time of *V. parahaemolyticus* in the exponential phase is 9–13 minutes.

*V. parahaemolyticus* is found in estuarine environments throughout the world, and can be isolated from estuarine waters during the summer but not in the winter. However, the vibrio can be isolated from the sediment when the temperature is less than 10˚C, and it probably survives in the sediment during the winter. It is unable to survive in ocean water. *V. parahaemolyticus* adheres to chitin through production of chitinase; in this way it can colonize zoo-plankton and the surface of shellfish. It also colonizes the digestive tract of shellfish. *V. parahaemolyticus* is often found during the summer in fresh water or streams, but especially in brackish water. It is unknown whether *V. parahaemolyticus* enter into a viable but non-culturable state in cold environments; such a state has been reported for *V. cholerae* and *V. vulnificus*.

### 1.3 Nature of infection

#### 1.3.1 Clinical manifestations

*V. parahaemolyticus* produces gastroenteritis in humans, and signs and symptoms usually occur approximately 12 hours after ingestion of contaminated food. The outstanding features are severe abdominal pain and diarrhea. The spectrum of manifestations may be very mild with only a few loose stools, or may be severe with stools containing blood and mucus. Recovery and disappearance of vibrio from stools are usually complete within a few days. Person-to-person transmission has not been observed. The mortality rate of *V. parahaemolyticus* infection is very low. *V. parahaemolyticus* is occasionally isolated from infected skin or soft tissue lesions of fish handlers, but its etiologic role in these infections is unknown.

The minimal infectious dose of *V. parahaemolyticus* in man varies with strain and individual, but was $10^5$–$10^8$ CFU in human volunteer experiments (Takikawa, 1958; Sanyal and Sen, 1974).
1.3.2 Virulence factors

Kato et al. (1965) found that strains of *V. parahaemolyticus* isolated from patients with gastroenteritis were hemolytic whereas those from seafish and sea environment were predominantly non-hemolytic on a modified brain heart infusion agar. A thermostable extracellular substance is responsible for the hemolytic reaction. This hemolysis is called the Kanagawa reaction. Sakazaki et al. (1986b) found that 96% of 2720 strains from patients with gastroenteritis were Kanagawa-positive, while only 1% of 650 environmental strains were Kanagawa-positive. Feeding Kanagawa-negative strains to 15 adult human volunteers failed to induce clinical signs (Sakazaki et al., 1968b). The hemolysin responsible for the Kanagawa reaction is a thermostable direct hemolysin (TDH). It is probable that TDH plays a role in the pathogenesis of the vibrio gastroenteritis.

Some outbreaks of gastroenteritis may be associated with Kanagawa-negative *V. parahaemolyticus*; Honda et al. (1988) demonstrated that those Kanagawa-negative strains produced a TDH-related hemolysin (TRH) but not TDH. Shirai et al. (1990) and Kishishita et al. (1992) also found that not only TDH-positive but also TRH-positive vibrios were associated with gastroenteritis. Further studies have shown that many clinical strains produce both hemolysins, or at least possess the genes that encode them.

A variety of pili and other potential colonization factors have been described for *V. parahaemolyticus*, but substantial studies have indicated that candidate adhesins are lacking.

1.3.3 Toxins

The TDH is a 21-kDa protein that is not affected by heating at 100°C at pH 6.0. It is a pore-forming toxin (Honda et al., 1992) and expresses hemolytic activity, cytotoxicity, enterotoxicity, and cardiotoxicity. In the hemolytic reaction the TDH is strongly active against erythrocytes of dogs, mice, rats, and humans, weakly active against those of rabbits and sheep, and inactive against horse erythrocytes. Nishibuchi et al. (1992) reported that a TDH-positive strain caused fluid accumulation in ligated ileal loops in rabbits whereas its TDH-negative mutant failed to do so. It was indicated that G_{T1b} is the intestinal receptor for TDH, but work by Yoh et al. (1995) suggested the presence of other unknown receptors.

Although TRH is similar to TDH in biological, immunological, and physicochemical properties, it is thermostable and differs in activity on erythrocytes (Honda et al., 1988). The TRH is linked epidemiologically to gastroenteritis (Nishibuchi, 1990), but the activity of this toxin is still uncertain.

1.4 Prevalence in food

Coastal fish and shellfish are usually contaminated with *V. parahaemolyticus* during the summer, and may contain high levels of the organism. The vibrios on the surface of fish or shellfish do not proliferate when the temperature is kept below 10°C, but at 20°C the number of vibrios increases very rapidly. Asakawa et al. (1974) reported that about 10–100 cells of *V. parahaemolyticus* were found on the surface of coastal fish just after landing, but if they were kept at atmospheric temperatures in the summer
the numbers became more than $10^6$ within a few hours. Vibrios are not found on market fish in the winter.

Oysters are often contaminated with *V. parahaemolyticus*. In Japan, they are seldom implicated in vibrio gastroenteritis because they are usually eaten during the cold season. However, in the US this species is a leading cause of gastroenteritis associated with raw shellfish consumption, particularly during the summer months (Hlady, 1997). Boiled or roast fish may sometimes be incriminated when the food has been contaminated from raw fish.

### 1.5 Foods most often associated with human infection

Gastroenteritis due to *V. parahaemolyticus* is almost always associated with seafood. Raw fish meat and shellfish are the most important sources of the disease in Japan, where the high incidence is without doubt due to the national custom of eating raw fish and fish products. However, the lack of correlation of the Kanagawa reaction between isolates from patients and from implicated seafoods is one of the puzzling *V. parahaemolyticus* problems.

Seafoods responsible for illness vary with local eating habits in different countries. Any kind of seafish served as ‘sushi’ and ‘sashimi’ can possibly cause gastroenteritis. The vibrio may be killed in 0.5% acetic acid within several minutes, but raw fish or shellfish treated with vinegar, which is commonly done in Japan, often transmit vibrio gastroenteritis. Vibrio gastroenteritis has sometimes been associated with consumption of raw vegetables which have been contaminated with the vibrio through kitchen utensils.

*V. parahaemolyticus* infection may be less important in European countries. Infection is unlikely in those countries where people are not in the habit of eating raw fish meat. Nevertheless, cases of gastroenteritis due to this vibrio have been reported in East European countries, the UK and Africa, in addition to the US. In these countries seafoods are usually cooked shortly before consumption, but crab and shrimp – the seafoods most often associated with vibrio infection – are usually handled after cooking, which may result in cross-contamination from other sources. It is probable that *V. parahaemolyticus* is a frequent cause of diarrheal disease in developing countries where people have a poor water supply and poor sanitary conditions. In such countries, waterborne infection with the vibrio could be considered.

### 1.6 Principles of detection

Although several selective agar media have been devised, TCBS (thiosulfate citrate bile salts sucrose) agar is the recommended medium for isolation of *V. parahaemolyticus*. This species forms green colonies on TCBS agar, since it does not ferment sucrose. However, routine use of TCBS agar for plating of stool specimens may not be cost-effective. MacConkey agar containing additional 0.5% NaCl is convenient for routine culturing of diarrheal stools.

Enrichment culture is used for detection of the vibrio in foods and from marine sources. Polymyxin salt broth with a pH of 7.6 and containing 2% NaCl and 50 µg/ml
of polymyxin B may yield selective growth of *V. parahaemolyticus*. Alkaline peptone water has also been used in many laboratories for enrichment. It should be noted that some factor(s) in shellfish may inhibit growth of vibrios. It is recommended that shellfish be cut into small pieces but not homogenized. After the pieces have been put into the enrichment broth it should be shaken vigorously and the pieces removed from the broth with forceps. However, enrichment culturing of seafood that is suspected of causing an outbreak may not be helpful, because most of the isolates from the food will be Kanagawa-negative – in contrast to isolates from patients.

The colonial appearance of *V. parahaemolyticus* on TCBS agar is so typical that provisional identification of isolates from stool specimens may be made directly on the plate. However, isolates from marine sources must be further examined in order to differentiate them from related organisms. The addition of 1% NaCl to media for biochemical tests is essential for positive reactions. Miyamoto et al. (1989) developed a rapid and sensitive assay for *V. parahaemolyticus* in seafoods using a fluorogenic method.

Wagatsuma agar has been used to test for the Kanagawa reaction. However, the test with Wagatsuma agar is difficult to perform due to problems with the preparation of this specialized medium. Honda et al. (1989) reported an enzyme-linked immunosorbent assay for detection of THD-producing vibrios, and Bej et al. (1999) described a multiplex PCR procedure to detect both hemolysin genes.

Several molecular approaches to detect Kanagawa-positive vibrios have been developed. DNA probes and oligonucleotide probes specific for the genes encoding TDH (*tdh*) or TRH (*trh*) were described by Nishibuchi et al. (1985, 1986) and Yamamoto et al. (1992). In their methods, however, the probes also hybridize with *tdh* genes in some strains of *V. cholerae* non-O1, *V. hollisae* and *V. mimicus*. Lee and Pan (1993) reported a polymerase chain reaction technique using oligonucleotide primers derived from the nucleotide sequence of the *tdh* gene. Also, Bej et al. (1999) described a multiplex PCR procedure to detect both hemolytic genes.

Strains capable of producing urease have been found to be associated with many clinical cases. On the west coast of the US, the majority of Kanagawa-positive strains were urease positive (Kaysner et al., 1994). Upon further investigation these common serogroups were also found to contain both the *tdh* and *trh* genes. Subsequently, clinical isolates from other areas of the US, and also Asia, were found to be urease positive. *V. parahaemolyticus* possessing the *trh* gene nearly always produces urease (Okuda et al., 1997).

Serotyping of *V. parahaemolyticus* isolates can be performed by slide agglutination tests using O and K antisera. In outbreaks of *V. parahaemolyticus* infections it is, however, seldom that the serovar identified in the patients is also detected in the incriminated seafood. Therefore, serotyping of isolates from seafoods and marine sources is generally not helpful. Several serotypes have been found in a majority of patients, i.e. serogroups O4:K12 and O1:K56 on the west coast of the US. It has recently been found that clinical strains in Asian countries are predominantly serogroup O3:K6 (DePaola et al., 2003). This serogroup also caused the largest reported oyster-associated outbreak in the US, in 1998 (Daniels et al., 2000).
2 Vibrio vulnificus

2.1 Historical aspects

Hollis et al. (1976) studied a biogroup of marine vibrio referred to as the ‘lactose-fermenting’ or ‘Lac⁺’ vibrio. This vibrio was subsequently given the scientific name Vibrio vulnificus by Farmer (1979).

2.2 Characteristics

2.2.1 Classification and phenotypic characteristics

This vibrio can grow on/in ordinary media containing 1–6 % NaCl, but not at 0 % or 8 %. Most strains are encapsulated. This species is divided into two biogroups; biogroup 1 involves both clinical and environmental strains and is indole-positive, while biogroup 2 includes only strains that are pathogenic to eels and is indole-negative. Most strains do not ferment sucrrose, but an occasional strain may be fermentative. Cellobiose is fermented by all strains, differentiating this species from V. parahaemolyticus. For phenotypic characteristics of V. vulnificus see Table 5.1.

Sero logically, 18 O groups were defined in this species by Shimada and Sakazaki (1984). Recent studies found that multiple strains may be present in one sample of shellfish, based on pulsed field gel electrophoresis and ribotype patterns (Tamplin et al., 1996).

2.2.2 Virulence factors

Although a variety of potential virulence factors, such as capsule (Kreger et al., 1981), cytotoxin (Gray and Kreger, 1987), collagenase (Smith and Merkel, 1982), siderophore (Simpson and Oliver, 1983) and protease (Kothary and Kreger, 1985) have been reported in clinical and environmental strains of V. vulnificus, the role of the capsule in the pathogenesis of human infection appears to be clearly established. Encapsulated cells are virulent to mice, are resistant to the bacteriocidal activity of human serum and to phagocytosis by macrophages, and are able to grow in iron-deficient media; these characteristics distinguish them from unencapsulated cells (Tamplin et al., 1985). In individuals, especially those with hepatic cirrhosis, hepatoma or hemochromatosis, the vibrio induces septicemia. It has been suggested that lack of complement, functional defects of the reticuloendothelial system or the presence of free Fe²⁺ in serum, or all three, are important factors in development of septicemia. However, Biosca et al. (1993) suggested that the production of capsule by strains of biotype 2 is not associated with illness in eels.

The cytolysin (hemolysis), proteases, and collagenase are considered tissue-damaging factors in wound infections with V. vulnificus, but the production of these factors has been found in strains that are either virulent or avirulent in mice (Morris et al., 1987).

2.2.3 Ability to survive and grow in the environment

V. vulnificus is widely distributed in the estuarine environment, but it can be isolated only during the warmer months. Although unable to be cultured in the cold season,
the vibrios are likely to be present but are in an apparently viable but non-culturable state (Oliver and Wanucha, 1989).

### 2.3 Nature of infection

#### 2.3.1 Human infections

In humans this species is associated with two disease syndromes, primary septicemia and wound infection. Septicemia caused by *V. vulnificus* is very serious, with a case/fatality rate of about 50%. Progression of illness can be very rapid, from asymptomatic to death within 24 hours. The important risk factor is pre-existing hepatic disease, especially cirrhosis, and about 75% of patients with primary septicemia caused by *V. vulnificus* have hepatic disease leading to increased levels of iron in serum (Blake *et al.*, 1979).

Wound infection with *V. vulnificus* usually develops after trauma and exposure to marine environment, and progresses rapidly. The infection is most commonly present as a cellulitis with a case/fatality rate of 7%. Approximately one-third of the patients with wound infection may have underlying disease.

Other infectious diseases from which *V. vulnificus* have been isolated include pneumonia and endometritis (Tison and Kelly, 1984). *V. vulnificus* has also been recovered from the stools of patients with diarrhea (Johnston *et al.*, 1986), but its etiological role in diarrhea has not been proven. Observations of large series of patients with *V. vulnificus* infections in Taiwan, Korea and the US have been published (Park *et al.*, 1991; Chuang *et al.*, 1992; Hlady, 1997).

It has been suggested that biogroup 2 is not associated with human infection. However, Veenstra *et al.* (1992) reported the implication of a strain of biogroup 2 in a patient with septicemia.

#### 2.3.2 Reservoirs and transmission

Principal reservoirs for *V. vulnificus* are coastal seawater and brackish water. During summer months, filterfeeding shellfish, such as oysters and clams, can contain high levels of this species. *V. vulnificus* is not sewage-associated.

### 2.4 Prevalence in foods

Oliver (1981) described that oysters contaminated with *V. vulnificus* and held for 24 hours on ice showed a 3-log decrease in cells. At the most common storage temperature of 8°C there can be up to a 2-log increase, and at 20°C even higher increases. Oliver (1981) also found that homogenization of oyster meat may lead to release of lethal factors that are very detrimental to the vibrios when combined with cold storage.

### 2.5 Foods most often associated with human infections

Foodborne *V. vulnificus* infections have been associated almost exclusively with raw seafoods, especially oysters.
2.6 Principles of detection

*V. vulnificus* grows well on TCBS agar. Blood agar is most commonly used in the clinical laboratory, since blood and wounds are the usual sources of this organism. Two agar media, cellobiose-polymyxin B-colistin agar (CPC; Massad and Oliver, 1987) and sodium dodecyl sulfate-polymyxin B-sucrose agar (Bryant *et al.*, 1987) have been developed for the isolation and enumeration of *V. vulnificus* in shellfish and environmental samples. A recent modification of CPC leaving out the polymyxin B, called CC agar (Hoy and Dalsgaard, 1998), has also been used effectively for isolation. The samples require enrichment using alkaline peptone water before plating. *V. vulnificus* has been detected by PCR (Brauns *et al.*, 1991; Hill *et al.*, 1991). A labeled DNA probe to detect *V. vulnificus* and a sandwich enzyme-linked immunosorbent assay for cytotoxin detection in environmental samples were reported by Wright *et al.* (1993), Parker and Lewis (1995) and DePaola *et al.* (1997). Additionally, DNA probes have been used to differentiate *V. vulnificus* from the phenotypically similar *V. parahaemolyticus* (Wright *et al.*, 1993; Kaysner *et al.*, 1994; Gooch *et al.*, 2001).

3 Vibrio cholerae

3.1 Historical aspects

Cholera was originally endemic in the delta of the River Ganges in eastern India, but before 1960 it had expanded into six pandemics that affected most of the world. There was another focus of a cholera-like disease, which was called El Tor cholera, in Cerobes, Indonesia; this disease has been spreading to large parts of the world since 1961 and the expansion is continuing.

The causative agent of the seventh cholera pandemic is a different serovar of the same serogroup (O1) as the original cholera vibrio, but in late 1992 the Indian subcontinent experienced an epidemic of cholera caused by a new type of O group designated O139. This is the first serogroup other than O1 to have caused a large-scale epidemic. There have been no reports of spread of this serogroup to other continents (Albert, 1994).

3.2 Characteristics

3.2.1 Classification and phenotypic characterization

So-called NAG (non-agglutinable) vibrios or NCV (non-cholera vibrios) are now classified into *V. cholerae*, in which the cholera vibrio is assigned to O group 1 (Sakazaki *et al.*, 1967).

*V. cholerae* has a single polar, sheathed flagellum. Many strains of *V. cholerae* non-O1 are encapsulated. There are reports, although rare, of cholera-like diarrheal illnesses and septicemic infections caused by non-O1/O139 serogroups.

Growth occurs between pH 6.0 and 9.6, and between 15˚C and 42˚C. Growth is supported by peptone water without additional salt. Phenotypic characteristics of
**Foodborne Infections**

*V. cholerae* are shown in Table 5.1. *V. mimicus* is a closely related species (Table 5.1) reportedly causing human gastroenteritis and sharing many characteristics with *V. cholerae*, including the ability to grow in media without added NaCl (Davis *et al.*, 1981); it can be differentiated from *V. cholerae* on TCBS agar by its lack of sucrose fermentation.

*V. cholerae* O1 is divided into two biovars, the classical and El Tor (eltor), but the classical biovar has become exceedingly rare. The differential characteristics of the two biovars are shown in Table 5.2. The differentiation was originally based on hemolytic activity on sheep red blood cells, but the hemolysis test does not always give reproducible results. A modified CAMP test in which the synergistic hemolysis is associated with eltor biovar and non-O1 strains was described by Lesmanna *et al.* (1994). Non-O1 serogroups are predominantly hemolytic. *V. cholerae* is inhibited by the vibriostatic compound O/129, but O1 strains resistant to this compound are increasingly found; O139 strains are resistant to O/129.

*V. cholerae* is divided into a number of O groups. So far, 140 O groups have been established (Sakazaki *et al.*, 1970; Shimada *et al.*, 1994). There are three O antigenic variants of *V. cholerae* O1 named Ogawa, Inaba and Hikojima, based on their three O antigen factors. The Ogawa strains are the original form, and the Inaba strains are mutants that have lost an Ogawa-specific factor. The rare Hikojima variants are regarded as a stable intermediate form between the Ogawa and Inaba variants (Sakazaki and Tamura, 1971). The conversion from Ogawa to Inaba is irreversible.

### 3.2.2 Virulence factors

#### 3.2.2.1 Colonization factor

The involvement of a variety of pili has been suggested for intestinal colonization by *V. cholerae* O1 and O139, but a long filamentous pilus is essential for colonization (Taylor *et al.*, 1987). This pilus is named toxin-coregulated pilus (TCP). Outer membrane proteins (OMP) also appear important for colonization. One possible colonization factor of OMP is the accessory colonization factor (acf) (Peterson and Mekalanos, 1988). An adhesin called OmpU was shown to mediate adherence to human epithelial cells (Sperandio *et al.*, 1995). Franzon *et al.* (1993) reported that hemagglutinin is a possible colonization factor. The capsular polysaccharide mediates adherence to epithelial cells in *V. cholerae* non-O1 (Johnson *et al.*, 1991). Such a capsule

<table>
<thead>
<tr>
<th>Test</th>
<th>Classical</th>
<th>El tor</th>
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</thead>
<tbody>
<tr>
<td>Hemolysis of sheep erythrocytes</td>
<td>–</td>
<td>+(^a)</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Chicken erythrocyte agglutination</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Polymyxin B, 50 IU</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Mukerjee’s phage IV</td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

\(^a\) Recent isolates are sometimes negative.
would also promote septicemia caused by non-O vibrios in susceptible hosts. Motility may help *V. cholerae* reach the intestinal mucosa.

### 3.2.2.2 Detachment

The ability to detach from host cells could be important for allowing bacteria to leave mucosal cells and reattach to newly formed mucosal cells. *V. cholerae* produces mucinase that degrades different types of protein. It allows *V. cholerae* to detach from cultured human intestinal epithelial cells.

### 3.2.2.3 Cholera toxin (CT)

Cholera toxin, a heat-labile protein, is responsible for the massive diarrhea characteristics of cholera. CT consists of one subunit A and five identical subunits B. Excreted CT attaches through subunit B to the surface of mucosal cells by binding GMI gangliosides. Once CT is bound, subunit A is released and translocated into the host cell. The intracellular target of CT is adenyl cyclase, which mediates the transformation of ATP to cyclic AMP (c-AMP) with subsequent increase in intracellular levels of c-AMP. The increased intracellular c-AMP concentration leads to increased chloride secretion by intestinal crypt cells and to decreased NaCl-coupled absorption by villus cells, resulting in water flow into the lumen and diarrhea (Finkelstein et al., 1992). CT or immunoologically related enterotoxins are also demonstrated in some non-O1 strains. The presence of the CT gene or production of CT in vitro is a trait used by health officials to assess the possible virulence of strains isolated from environmental samples or food products.

Two new enterotoxins of *V. cholerae* were described by Fasano et al. (1991) and Trucksess et al. (1993). One of them, zona occludens toxin (Zot), increases the permeability of the small intestine mucosa by disrupting the intracellular tight junctions that bind mucosal cells together. The other is an accessory cholera toxin (Ace), which causes significant fluid accumulation in rabbit ileal loops. Genes encoding CT (*ctx*) are located on a transposon, and only strains that acquire this transposon produce toxin. The *zot* and *ace* genes are close to the *ctx* on the chromosome and may be on the same transposon, thus suggesting that *ctx*, *zot* and *ace* genes constitute a virulence cassette. On the other hand, Sanyal et al. (1983) reported a toxin in CT-negative O1 strains. This toxin was named ‘new cholera toxin’, and was shown to cause diarrhea in human volunteers (Saha and Sanyal, 1990).

The hemolysin of *V. cholerae* is a cytotoxin that causes fluid accumulation in rabbit ileal loops. In contrast to the fluid produced with CT; however, the accumulated fluid produced in response to hemolysin is invariably bloody and contains mucus (Ichinose et al., 1987). Occasional strains of *V. cholerae* also produce other toxins, including a Shiga toxin in O1 strains (O’Brien et al., 1984) and the thermostable enterotoxin (ST) of *E. coli* in some non-O1 strains (Morris, 1990). Genes encoding the THD-related hemolysin (TRH) produced by some Kanagawa-negative strains of *V. parahaemolyticus* were found on a plasmid in some non-O1 strains (Honda et al., 1986).

Environmental O1 isolates outside of epidemic areas are almost always CT-negative. However, those CT-negative strains, as well as non-O1 strains, may cause not only diarrhea but also extraintestinal infections.
3.2.3 Ability to survive and grow in the environment

*V. cholerae* O1 does not survive in fresh water for more than 7–10 days, particularly during the warm season; its survival in seawater is longer. *V. cholerae* produces chitinase and it preferably colonizes the surface of shellfish and zooplanktons, therefore the estuarine environment represents an ideal setting for survival and persistence of *V. cholerae*. Spira *et al.* (1981) reported that water hyacinths from Bangladesh waters were shown to be colonized by *V. cholerae* and to promote its growth. Colwell and Huq (1994) noticed a survival strategy of *V. cholerae* in the environment in which it remained viable but was not culturable. The Rugose variant of *V. cholerae* is also assumed to have a resistant form (Morris *et al.*, 1993).

3.3 Nature of the infection

3.3.1 Clinical manifestations

In typical cholera there is profuse diarrhea, with large volumes of so-called rice-water stool passed painlessly. This can amount to twice the body weight within 4–6 days. Gastric disturbances, particularly subacidity and gastrectomy, are risk factors in severe cholera. If untreated there will be prostration with symptoms of severe dehydration, and death can occur very quickly after onset of symptoms. The disease is easily treated by monitoring body fluid loss combined with rehydration.

Cholera is usually a disease of the lower socioeconomic groups because of their poor hygienic standards. Most patients have either a mild diarrhea or no symptoms at all. The ratio of severe to mild asymptomatic cases is between 1:5 and 1:10 for classical cholera, but only 1:25 to 1:100 for eltor cholera. However, during the recent epidemic in Peru there were over 70% severe cases and a 60% case/fatality rate (Pan American Health Organization, 1993). In addition, it was reported that the vibrios isolated in South and Central America can be clearly distinguished genetically from strains causing the seventh pandemic. The World Health Organization estimates that there are greater than 150,000 cases of cholera per year on multiple continents.

3.3.2 Reservoirs and transmission

Excretion of the vibrios by infected persons usually lasts for only a few days, and carriers are rarely found to harbor the vibrios for a long period. Cholera is more likely to occur in families with asymptomatically infected breast-fed infants, who themselves are protected against the illness by maternal antibodies. Domestic animals in epidemic areas often carry O1 vibrios (Sanyal *et al.*, 1974).

Cholera is not spread by direct contact. The most important mode of spread is through the environment, particularly contaminated water (Hughes *et al.*, 1982). Raw shellfish are also important sources of infection. Not only the surface but also the digestive tracts of shellfish are colonized by *V. cholerae*; the digestive tract is infected through ingestion of zooplankton in which the vibrios were absorbed. *V. cholerae* persists for many weeks in shellfish, and can thus maintain its lifecycle without continuous contamination with human feces containing the vibrio.
3.4 Prevalence in foods and water

The presence of CT-positive strains of *V. cholerae* is not always associated with fecal contamination from cholera patients. Water may also become contaminated with *V. cholerae* during household storage. In developing countries, beverages are potential vehicles of cholera transmission. Beverages containing ice may be specifically incriminated because the ice is made from contaminated municipal water. Chlorination of public water systems is an effective means of controlling epidemics. *V. cholerae* survives for between 2 and 14 days on most foods, and survival is increased when foods are cooked before contamination. A short survival period is common in acidic foods such as fruits, whereas survival may be several weeks in cooked and raw vegetables. A pH of 5.0 or lower has been found to be very detrimental to *V. cholerae* survival.

3.5 Foods and water most often associated with infection

A significantly increased risk of infection is associated with use of contaminated water for food preparation, bathing or washing. Bathing in contaminated surface water may be particularly risky in Moslem communities, since it is common to rinse the mouth with water. Seafoods, particularly shellfish, may acquire the vibrio from environmental sources, and may serve as a vehicle in epidemic cholera.

3.6 Principles of detection

3.6.1 Isolation

Numerous agar media have been devised for the isolation of *V. cholerae*, but TCBS agar is probably the most widely used; on this medium *V. cholerae* appears as yellow colonies due to sucrose fermentation. For enrichment culture alkaline peptone water (pH 8.6–9.0) is used; it supports good growth of *V. cholerae*, but the incubation period is best limited to 8 hours to prevent overgrowth with other organisms. Stool specimens should be collected as soon as possible, and inoculated onto isolation agar plates. For formed feces, enrichment culture may be necessary. Water samples are passed through a membrane filter and the filter disk is then placed in alkaline peptone water. Moore's swabs have also been used successfully to isolate *V. cholerae*. Food samples are blended in alkaline peptone water and then treated the same way as water samples. Shellfish should be cut into small pieces but not homogenized. After putting the pieces into enrichment broth and shaking vigorously, the pieces are removed from the broth because factors in those seafoods may inhibit the vibrios. DePaola *et al.* (1987) recommended incubation of a separate portion of the sample in alkaline peptone water at 42°C. For the detection of vibrios that are in a dormant state, Huq *et al.* (1990) reported a method using fluorescence microscopy.

The rapid diagnosis of cholera is of great importance; therefore suspect colonies from foods related to an outbreak should be tested first for agglutination directly with *V. cholerae* O1 and O139 antisera.
3.6.2 Direct antigen detection methods
There are enough O antigens in rice-water stools of cholera patients to be agglutinated with O antibodies. A coagglutination test using monoclonal antibodies to an O1-specific epitope has been developed recently. An antigen capture test using a colloidal gold-based colorimetric immunoassay has been reported by Hasan et al. (1994).

3.6.3 Toxin assays
Various modifications of an ELISA using purified G\textsubscript{MI} ganglioside receptor as the capture molecule are now commonly used to assay CT. Almeida et al. (1990) reported, however, that the latex agglutination assay to detect CT was less complicated and less time-consuming than the ELISA. In addition, a variety of molecular approaches to toxin detection have been developed. PCR has been used for the detection of the ctx gene (Fields et al., 1992), and was shown to be more sensitive than a bead ELISA for detecting CT in stool specimens (Ramamurthy et al., 1993).

Acknowledgment
The passing of Riichi Sakazaki, an internationally known microbiologist and a genuinely gracious scientist, saddens us. I, Charles Kayser, first met Dr Sakazaki in 1973, when he toured the US to provide information to many public health laboratories on the identification of \textit{V. parahaemolyticus}. This was after the first major outbreak in the US. Over the years and having had the opportunity to meet him at various scientific meetings, he was always the gracious individual and always interested to hear of work in other laboratories. This excellent scientist will be missed.

Bibliography