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Vibrio spp

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(materials from Dean Cliver)

Genus *Vibrio*:

Vibrio spp. are part of the normal flora in marine habitat, and many of them were identified as the most serious pathogens in fish and shellfish marine aquaculture worldwide.
Gram-negative, non-spore-forming bacilli, 0.5–0.8 μm diameter, 1.4–2.6 μm long; usually motile by a single polar flagellum
Facultative; stimulated by NaCl or require it
Many species and subspecies known
Those of interest in connection with human disease seem to have a natural habitat in brackish water and saltwater.

1. *Vibrio parahaemolyticus*

1.1 History

Vibrio parahaemolyticus is considered to be the causative agent in 50–70% of all cases of diarrhea associated with the consumption of fishery products in the summer months in China.
Three *Vibrio* outbreak reports in Japan in 1953 and 1960; leading cause of foodborne disease in Japan.
Associated with seafood worldwide. CDC reports 25 outbreaks comprising 613 cases (0 deaths) in the U.S., 1998–2002; vehicles were: shellfish (20), poultry (1), "complex" (2), unknown (2).
CDC estimates 5,122 cases of foodborne vibriosis, other than cholera or *V. vulnificus* infection, in the U.S./year, with 13 deaths.

1.2 Characteristics

Pathogenic strains are "Kanagawa-positive": produce hemolysis on a specially compounded (Wagatsuma, pH 8) blood agar medium.
Optimum growth in 2–4% NaCl, grows at 8%, but not 10% NaCl.
pH growth range 7.5–8.6 optimum (survives pH 4.8–11)
Temperature limits are >10ºC–42ºC or 44ºC; will grow in seafood held at permissive temperatures.

1.3 Infection in man and animals

Infection probably requires ingestion of $>10^5$ cells (ingested with seafood).
Incubation 4–30 hr (usually 12–24 hr)
Watery diarrhea with abdominal cramps in most cases, sometime with nausea, vomiting, fever, and headache; rarely, dysentery-like illness; duration 1–7 days
Not communicated person-to-person

Infections of nonhuman animals not mentioned
During cold weather, organism is found in marine sediment.
During warm weather, occur in seawater (normal flora) and seafoods.
Foods most often associated with human infections are seafoods, both shellfish and finfish; organism is killed by cooking or by irradiation. In a study in China, a total of 83 shellfish samples from two local retail sources, 38 samples were positive, and *V. parahaemolyticus* were recovered from all six types of shellfish in the stores. In Mexico, more than 1230 cases of gastroenteritis were reported. All cases were attributed to the consumption of raw or undercooked shrimp collected from the Huizache-Caimanero lagunary system.

1.4 Principles of detection of *V. parahaemolyticus*

Homogenate of seafood sample (finfish surface tissues, gut, gills; soft tissues of molluscs; at least gut and gills of shrimp) diluted serially and mixed with equal volume of enrichment medium (choice of at least three), incubated overnight at 35ºC±2ºC; streaked onto selective agar from most dilute enrichment tube that shows growth, incubated overnight at 35ºC±2ºC; proceed with biochemical identification (includes differentiation from *V. vulnificus*); enrichment tubes can be scored for most probable number quantification.
Alternately, direct quantification can be done on hydrophobic grid membrane, using a 1:10 homogenate of sample in peptone-Tween-salt diluent; filter is incubated 4 hr at 35ºC on one agar medium, then 18–20 hr at 42ºC on another; *V. parahaemolyticus* colonies are green to blue ─others are yellow. Serologic classification is based on O (somatic) and K (capsular) antigens.
Unfortunately, there are several problems concerning detection of V. *parahaemolyticus* in seafood using culture methods, and it is recommended to use new techniques such as the PCR method. Real-time PCR offers a rapid and quantitative analysis for the detection of foodborne pathogens.

2. *Vibrio cholerae*

2.1 History

Causes **cholera**
Waterborne transmission is widespread in the developing world: most outbreaks in the 19th and first half of the 20th centuries occurred in Asia and involved “classical” *V. cholerae*, serogroup O1; causes pandemics.
From 1961, the El Tor biotype of serogroup O1 has predominated and has occurred in many parts of the world. Another serogroup, O139, has arisen in Asia in 1992 and is causing illnesses and deaths in at least seven Asian countries.
In January of 1991, an outbreak due to serogroup O1, biotype El Tor, began in Peru and spread through much of Latin America
Essentially all cholera in the U.S. has been serogroup O1, biotype El Tor Inaba, transmitted by seafood (eaten raw or undercooked) from the Louisiana and Texas Gulf Coasts

2.2 Characteristics

Grows in the range of 15ºC–42ºC, optimum 30ºC–37ºC.

pH range for growth is 6–10; tolerates alkaline conditions but is acid-sensitive.
Does not require salt, but will grow in the presence of up 6% NaCl.
Virulence depends on production of ill-defined colonization factors and of cholera toxin (see below).
Serogroups other than O1 and O139 are fairly widespread; some of these cause diarrhea, but usually not cholera; there are also O1 strains that do not produce cholera toxin and therefore do not produce the disease.

2.3 Infections in man

Infectious dose is apparently undetermined; infection is peroral.
Incubation period is a few hours to 5 days, usually 2–3 days.
Sudden onset of profuse, painless, watery diarrhea ("rice-water stools"), occasional vomiting; in untreated cases, dehydration may lead to circulatory collapse, acidosis, hypoglycemia in children, renal failure, and death; treatment consists principally of rehydration (oral or IV), but antibiotics may be useful; fatality rate with proper treatment <1%.
Survivors are immune, but not for life, to the same *V. cholerae* type.
The cholera enterotoxin is a complex protein that increases cyclic AMP in interstitial cells, leading to loss of fluid and electrolytes into the intestinal lumen.
During 1998–2002, CDC recorded 0 foodborne cholera outbreaks in the U.S., and no waterborne cholera outbreaks for the years 2003–2004.
CAST report "best estimates" for foodborne cholera in the U.S. are 25–13,000 cases per year, with fewer than 2 deaths; the cost per case is estimated at $1000.
CDC estimates 49 cases of food borne cholera in the U.S./year, with no deaths.

2.4 Principles of detection of *V. cholerae*

Diagnosis in humans may be based on isolation of the organism or detection of the toxin (e.g., by ELISA) in patients' stools.
Food samples are enriched in alkaline peptone water at 35ºC or 42ºC; detection is by plating on a variety of media, some nonselective; identification is by reactions on Kligler iron agar, positive "string test" (lysis of cells in 0.5% sodium desoxycholate, 0.85% NaCl solution releases DNA, which forms a string when lifted from the suspension with a loop), serologic grouping and typing, and biotyping (based on susceptibility to a standard phage or to polymyxin-B).

3. *Vibrio vulnificus*

3.1 History

This organism and the diseases it causes seem to have been recognized first in 1979.
Because of high lethality, it is now regarded as an important foodborne disease hazard in the U.S., and possibly in other developed countries
For 1998–2002, CDC reports only one possible outbreak ("*Vibrio*, other"), perhaps because *V. vulnificus* most often causes individual (sporadic) cases.
The CAST report estimates (from Todd) 29,000 cases/year in the U.S., with 30 deaths and an average cost/case of $1275.

CDC estimates 47 foodborne illnesses yearly in the U.S., with 18 deaths.

3.2 Infection in man and animals

Vibrio vulnificus is an etiologic agent in severe human infection acquired through wounds or contaminated seafood.
The strains belonging to this species are divided into three biotypes according to their different biochemical and biological properties. Biotype 1 strains are pathogenic for humans; biotype 2, appear to be virulent for both humans and eels. A third biotype, indole-positive, causing wound infections and bacteremia in people handling St. Peter's fish (*Tilapia* spp.), has been isolated in Israel.
V. vulnificus has been detected in coastal and estuarine environments throughout the world in areas with warm seawater temperatures. During warm weather, when the sea temperature is high, greater frequency of infection has been observed. Shellfish may constitute one of the most hazardous foods if consumed raw or undercooked, as they can accumulate a high number of microorganisms from the overlying water.
People (usually men >40 years old) susceptible to foodborne infections are those with chronic liver disease, chronic alcoholism, or hemochromatosis, or who are immune suppressed; if they eat raw or undercooked seafood (especially oysters), they may become dramatically ill after 12 hours to 3 days, often with shock, distinctive bullous skin lesions, thrombocytopenia, and disseminated intravascular coagulation; death is frequent; tetracycline is the drug of choice.
Infected wounds acquired, say, while in coastal or estuarine waters or while shucking oysters may spread rapidly and become necrotic; victims of this form of infection may not have been previously abnormal; amputation of the affected limb may be necessary.
In China, an outbreak with high mortality within one week happened in intensive culture of *Trachinotus ovatus* (perch-like marine fish) around the Gulf Coast of Yangjiang city (Guangdong Province, China) from *Vibrio vulnificus.*
Infections of nonhuman animals not reported
Clams and oysters (eastern seacoast, U.S.), fairly common; among positive oysters, average level was 6×10^4 CFU/g; west coast distribution depends on water temperatures.
Seawater (eastern seacoast, U.S.), when positive, had <10 CFU/ml.

3.3 Principles of detection of *V. vulnificus*

Few details given: halophilic (grows in 6% but not 8% NaCl); ferments lactose but less frequently sucrose.
Mice "primed with iron dextran" are killed by intraperitoneal injection of *V. vulnificus.*
Detection methods are similar to those for *V. parahaemolyticus*; all media contain at least 0.5% NaCl; *V. vulnificus* grows at 6%, but not 8% NaCl.
Colonies of virulent *V. vulnificus* are opaque; in mice injected 2 hr previously with 250 μg of iron dextran/g of body weight, intraperitoneal injection of washed cells should yield an LD_{50} of <10 cells/mouse.

Summary

The genus *Vibrio* comprises species from brackish and marine waters; unlike many foodborne pathogens, these are not necessarily present in food as a result of human fecal contamination (possibly indirect human fecal contamination with *V. cholerae*).
At least three of these species are significant human pathogens, associated with seafoods in North America; all are easily killed by cooking the seafood.
V. parahaemolyticus is a worldwide problem with seafood, causes diarrheal illness that is not generally life-threatening. *V. cholerae* is usually waterborne elsewhere in the world; cholera is a life threatening disease if not properly treated, and still kills many people worldwide.
Foodborne *V. vulnificus* kills only a few people who have predisposing conditions; but it kills very quickly if diagnosis and treatment are delayed.

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