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***VIBRIO* SPP.**

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Genus *Vibrio*

Gram-negative, nonsporeforming 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

Vibrio cholerae

Historical aspects and contemporary problems

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

Characteristics of *V. cholerae*

Grows in the range of 15°–42°C, optimum 30°–37°C
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

Nature of the infection 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

Nature of toxic agent

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

Prevalence of *V. cholerae*

Water—brackish and marine waters may contain *V. cholerae*; the organisms is thought to multiply in zooplankton
Foods—*V. cholerae*, perhaps not the virulent variety, has been detected in seafood; although "shellfish" are mentioned, this probably includes crustacea, as well as molluscs

Foods/water most often associated with human infections

During 1993–1997, CDC recorded 1 foodborne cholera outbreak comprising 2 cases in the U.S. (vehicle unknown)
No waterborne cholera outbreaks were reported by CDC for the years 1999–2000
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 foodborne cholera in the U.S./year, with no deaths

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 35° or 42°C; 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)

Vibrio parahaemolyticus

Historical aspects and contemporary problems

Associated with seafood worldwide
Until recently, leading cause of foodborne disease in Japan
CDC reports 5 outbreaks comprising 40 cases (0 deaths) in the U.S.; 1993–1997 vehicles were: Chinese food (1), shellfish (1), multiple (1), unknown (2)
CAST estimates 9,000–29,360 U.S. cases and 30–360 deaths in the U.S. per year, with a cost per case (*"Vibrio cholerae/parahaemolyticus"*) of $1000
CDC estimates 5,122 cases of foodborne vibriosis, other than cholera or *V. vulnificus* infection, in the U.S./year, with 13 deaths

Characteristics of *V. parahaemolyticus*

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 >10°–42° or 44°C; will grow in seafood held at permissive temperatures

Nature of the 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

Reservoirs and transmission

During cold weather, organism is found in marine sediment
During warm weather, occurs 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

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 35°±2°C; streaked onto selective agar from most dilute enrichment tube that shows growth, incubated overnight at 35°±2°C; 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 35°C on one agar medium, then 18–20 hr at 42°C on another; *V. parahaemolyticus* colonies are green to blue—others are yellow

Serologic classification is based on O (somatic) and K (capsular) antigens

Vibrio vulnificus

Historical aspects and contemporary problems

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 1993–1997, CDC reports no outbreaks, 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

Characteristics 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*

Nature of the infection in man and animals

People (usually men >40 years old) susceptible to foodborne infections are those with chronic liver disease, chronic alcoholism, or hemochromatosis, or who are immunosuppressed; 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

Infections of nonhuman animals not reported

Prevalence of *V. vulnificus* in foods

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

Food most often associated with human infections: oysters

Principles of detection 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

Bibliography

Council for Agricultural Science and Technology (CAST) 1994. Foodborne pathogens: risks and consequences. Task Force Report No. 122, CAST, Ames, IA.

Heyman, D. L., ed. 2004. Control of Communicable Diseases Manual, 18th ed. American Public Health Association, Washington, DC.

International Commission on Microbiological Specifications for Foods. 1996. Vibrio cholerae. pp. 414–425. Vibrio parahaemolyticus. pp. 426–435. Vibrio vulnificus. pp. 436–439. *In* Microorganisms in Foods. 5. Characteristics of Microbial Pathogens. Blackie, London

Lee, S. H., D. A. Levy, G. F. Craun, M. J. Beach, and R. L. Calderon. 2002. Surveillance for waterborne-disease outbreaks — United States, 1999–2000. Morbid. Mortal. Wkly. Rep. 51(SS-8):1–52.

McLaughlin, J. C. 1995. *Vibrio*. pp. 465–476. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds), Manual of Clinical Microbiology, 6th ed. American Society for Microbiology Press, Washington, DC.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerging Infect. Dis. 5:607–625.

Oliver, J. D., and J. B. Kaper. 2001. *Vibrio* Species. pp. 263–300. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds., Food Microbiology: Fundamentals and Frontiers, 2d ed. ASM (American Society for Microbiology) Press, Washington, DC.

Olsen, S. J., L. C. MacKinnon, J. S. Goulding, and L. Slutsker. 2000. Surveillance for foodborne disease outbreaks — United States, 1993–1997. Morbid. Mortal. Weekly Rep. Surveill. Summ. 49(SS01):1–62.

Sakazaki, R. 2002. *Vibrio*. pp. 127–136. *In* D. O. Cliver and H. P. Riemann, eds. 2002. Foodborne Diseases, 2d ed., Academic Press, London.

Sakaziki, R., C. Kaysner, and C. Abeyta, Jr., 2006. *Vibrio* infections. pp. 185–204. *In* Riemann, H. P., and D. O. Cliver, eds. 2006. Foodborne Infections and Intoxications, 3d ed. Academic Press (Elsevier), London, Amsterdam.