Chapter 20: *Vibrio* spp.

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Potential Food Safety Hazard

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Vibrio spp.

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The genus *Vibrio* includes Gram-negative, oxidase-positive (except two species), rod or curved rod-shaped facultative anaerobes. Many *Vibrio* spp. are pathogenic to humans and have been implicated in food-borne disease. *Vibrio* spp. other than *V. cholerae* and *V. mimicus* do not grow in media that lack added sodium chloride, and are referred to as "halophilic" (Elliot et al., 1998)

Association of *Vibrio* spp. with different clinical syndromes[a,b].

	Clinical Syndrome				
Species	**Gastroenteritis**	**Wound Infection**	**Ear Infection**	**Primary Septicemia**	**Secondary Septicemia**
V. cholerae O1	+++	+			
V. cholerae non-O1	+++	++	+	+	+
V. mimicus	++		+		
V. fluvialis	++				
V. parahaemolyticus	+++	+	+		+
V. alginolyticus	(+)	++	++	+	
V. cincinnatiensis				+	
V. hollisae	++			+	
V. vulnificus	+	++		++	++
V. furnissii	(+)				
V. damsela		++			
V. metschnikovii	(+)			(+)	
V. carchariae		+			

[a]+++ = frequently reported, ++ = less common (6-100 reports); + = rare (1-5 reports), and (+) = association is unclear.
[b]Table taken from Pavia et al. (1989).

Vibrio cholerae

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V. *cholerae* was first described as the cause of cholera by Pacini in 1854. Pathogenic *V. cholerae* produces a heat-sensitive enterotoxin that causes the characteristic cholera symptoms, including "rice water stool." The species comprises several somatic (O) antigen groups, including O-group-1, which is associated with classical and El Tor biotypes. *V. cholerae* Ol may have several serotypes, including Inaba, Ogawa, and Hikojima. *V. cholerae* non-O1 (referred to in older literature as nonagglutinable or NAG vibrios) also can cause gastrointestinal disease, though typically less severe than that caused by *V. cholerae* O1 (Yamamoto et al., 1983). Serotype O139 is an exception, and produces classic cholera symptoms. This serotype was first identified in 1992 (CWG, 1933) as the cause of a new epidemic of cholera in India and Bangladesh. Non-O1 *V. cholerae* is found more readily in estuarin! e waters and seafood in the United States than is the Ol serogroup; however, the 0139 serogroup has not yet been found here. Because this species

can grow in media lacking sodium chloride, it is not considered a halophilic Vibrio, although traces of sodium ion are required for growth. The standard FDA method for recovery of *V. cholerae* is qualitative (presence/absence). Testing *V. cholerae* O1 and non-O1 isolates for production of cholera toxin is recommended.

Some diarrheal and otitis isolates, once thought to be atypical *V. cholerae* non-O1 (sucrose-negative), are now recognized as a separate species, *V. mimicus* (Davis et al., 1981; Shantera et al., 1983). Members of the species may produce cholera-like enterotoxins. *V. mimicus* can be identified by biochemical procedures used for the identification of *V. cholerae* (Elliot et al., 1998).

Vibrio parahaemolyticus

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V. *parahaemolyticus* is a halophilic bacterium found naturally in estuarine waters and animals. It was first described as the cause of gastroenteritis in Japan (Fujino et al., 1951) and was first found in the United States by Baross and Liston (1968) in the estuarine waters of Puget Sound. It has a worldwide distribution in estuarine and coastal environments and has been isolated from many species of fish, shellfish, and crustaceans. *V. parahaemolyticus* has been implicated in numerous outbreaks of seafood-borne gastroenteritis in the United States. Between 1971 and 1978, crab, oyster, shrimp, and lobster were implicated in 14 outbreaks, which may have resulted from the consumption of raw or insufficiently heated seafood or properly cooked seafood contaminated after cooking. The FDA method of enumeration uses an MPN format (Elliott et al., 1998).

Vibrio vulnificus

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V. *vulnificus* is a halophilic bacterium found in the estuarine environment and is similar phenotypically to *V. parahaemolyticus* (Oliver, 1989). The species was first described as "lactose-positive" because most strains ferment lactose and are *o*-nitrophenyl-β -D-galactosidase (ONPG) positive. It causes food-borne and wound disease, either of which may progress to rapidly fatal septicemia in individuals with liver disease (cirrhosis) or other underlying illnesses such as diabetes. Raw oysters are the major source of food-borne disease caused by *V. vulnificus*. The FDA method of enumeration uses an MPN series confirmed by biochemical testing or an immunological test, such as the ELISA, with monoclonal antibody to a species-specific intracellular antigen (Elliott et al., 1998).

Other *Vibrio* species

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Other halophilic *Vibrio* spp., including *V. fluvialis*, *V. hollisae*, *V. alginolyticus*, *V. furnissii*, and *V. metschnikovii*, have been associated with gastroenteritis and are present in estuarine environments along with other pathogenic and nonpathogenic species of *Vibrio*. *V. cincinnatiensis*, *V. damsela*, and *V. carchariae* have not been associated with gastroenteritis, but on rare occasions are pathogenic to humans (Table 20-1). *V. anguillarum*, *V. damsela*, and *V. carchariae* are pathogenic to fish. Biochemical testing is required for taxonomic speciation (Elliott et al., 1998).

Control Measures

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Hazards from *Vibrio* can be prevented by cooking seafood thoroughly and by preventing cross-contamination once the seafood is cooked. Freezing is ineffective in killing the bacteria (Ward et al., 1997).

If *V. parahaemolyticus* has produced the heat-stable Kanagawa hemolysin, some cooking procedures may not destroy the hemolysin (Bradshaw et al., 1984).

The risk of *V. vulnificus* infection can also be reduced by rapidly refrigerating oysters from the Gulf Coast during warm-weather months. Individuals in the "high risk" groups should not consume raw molluscan shellfish (Ward et al., 1997).

Guidelines

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FDA Guidelines

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FDA guidelines for *Vibrio* in fish.

ICMSF Recommended Microbial Limits

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Recommended microbiological limits for *V. parahaemolyticus* in fish (ICMSF, 1986).

Product	n[1]	c[2]	Bacteria/gram or/cm^2	
			m[3]	M[4]
Fresh and frozen fish and cold-smoked fish	5	2	10^2	10^3
Frozen raw crustaceans	5	1	10^2	10^3
Frozen cooked crustaceans	5	1	10^2	10^3
Cooked, chilled, and frozen crabmeat	10	1	10^2	10^3
Fresh and frozen bivalve molluscs	10	1	10^2	10^3

[1]Number of representative sample units.
[2]Maximum number of acceptable sample units with bacterial counts between m and M.
[3]Maximum recommended bacterial counts for good quality products.
[4]Maximum recommended bacterial counts for marginally acceptable quality products.

Plate counts below "m" are considered good quality. Plate counts between "m" and "M" are considered marginally acceptable quality, but can be accepted if the number of samples does not exceed "c." Plate counts at or above "M" are considered unacceptable quality (ICMSF, 1986).

Growth

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Limiting conditions for *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* growth.

Heat Resistance

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Heat resistance of *V. cholerae*.

Temp.		D-Value	Medium	Reference
(ºC)	(ºF)	(min.)		
48.9	120	9.17	Shrimp homogenate	Hinton and Grodner, 1985
49	120.2	8.15	Crabmeat	Shultz et al., 1984
54	129.2	5.02	Crabmeat	Shultz et al., 1984
54.4	129.9	0.43	Shrimp homogenate	Hinton and Grodner, 1985
60	140	2.65	Crabmeat	Shultz et al., 1984
60	140	0.39	Shrimp homogenate	Hinton and Grodner, 1985
65.5	149.9	0.32	Shrimp homogenate	Hinton and Grodner, 1985
66	150.8	1.60	Crabmeat	Shultz et al., 1984
66	150.8	1.22	Crayfish homogenate	Grodner and Hinton, 1985
71	159.8	0.30	Crabmeat	Shultz et al., 1984
71	159.8	0.30	Crayfish homogenate	Grodner and Hinton, 1985
71.1	160	0.31	Shrimp homogenate	Hinton and Grodner, 1985
76.7	170.1	0.30	Shrimp homogenate	Hinton and Grodner, 1985
77	170.6	0.27	Crayfish homogenate	Grodner and Hinton, 1985
82	179.6	0.27	Crayfish homogenate	Grodner and Hinton, 1985
82.2	180	0.28	Shrimp homogenate	Hinton and Grodner, 1985

Heat resistance of *V. parahaemolyticus*.

Temp.		D-Value	Medium	Reference
(ºC)	(ºF)	(min.)		
47	116.6	65.1	7.5% NaCl	Beuchat and Worthington, 1976
49	120.0	0.82	Clam homogenate	Delmore and Chrisley, 1979
51	123.8	0.66	Clam homogenate	Delmore and Chrisley, 1979

53	127.4	0.40	Clam homogenate	Delmore and Chrisley, 1979
55	131	0.29	Clam homogenate	Delmore and Chrisley, 1979

Heat resistance of *V. vulnificus*.

Temp.		D-Value	Medium	Reference
(ºC)	(ºF)	(min.)		
47	116.6	2.40	Buffered saline	Cook and Ruple, 1992
50	122	1.15	Buffered saline	Cook and Ruple, 1992

Analytical Procedures

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Compendium of Analytical Methods (HC)

Food sampling and preparation of sample homogenate (USFDA) Top

Definition of Terms (HC Appendix A); Collection of samples (HC Appendix B); Supplement to All Methods in the HC Compendium: General Microbiological Guidance (HC Appendix I) (Supplement to HC Appendix I: A. General Microbiological Guidance on Pre-warming of Broths in All Qualitative Methods in the Compendium) Top

V. cholerae, *V. parahaemolyticus*, *V. vulnificus*, and other *Vibrio* species (USFDA) Top

Detection of enterotoxigenic *Vibrio cholerae* in foods by the polymerase chain reaction (USFDA) Top

Detection of Halophilic Vibrio Species in Seafood (HC MFLP-37) Top

The isolation and identification of *Vibrio cholerae* 01 and non-01 from foods (HC MFLP-72) (pdf file) Top

The isolation and enumeration of *Vibrio vulnificus* from fish and seafoods (HC MFLP-73) (pdf file) Top

Other analytical procedures

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- *Vibrio cholerae* in oysters: Elevated temperature enrichment method (AOAC, 1995a).
- *Vibrio vulnificus*: Gas chromatographic identification method by microbial fatty acid profile (AOAC, 1995b).
- *V. vulnificus* in oysters: DNA probe (DePaola et al., 1997).

Commercial Test Products

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Commercial test products for *V. cholerae*.

Test Kit	Analytical Technique	Approx. Total Test Time[1]	Supplier
CHECK 3 Vibrio sp.	Chemical, visual detection	4-18 h	Contamination Sciences LLC Contact: Robert Steinhauser 4230 East Towne Blvd., Suite 191 Madison, WI 53704 Phone: 608/825-6125 E-mail: bsteinha@contam-sci.com Web: www.contam-sci.com
Chromogenic Vibrio [Presumptive differentiation of *V. parahaemolyticus* and *V. vulnificus*]	Chromogenic media	48 h	Biomedix Contact: Claver Bundac 1105 #F North Golden Springs Dr. Diamond Bar, CA 91765 Phone: 800/674-8648 #4282; 909/396-0244 E-mail: cb4biomedx@aol.com
ISO-GRID Method [For *Vibrio parahaemolyticus* count using VSP agar]	Membrane filtration with selective and differential culture medium using sucrose fermentation	24 h	QA Life Sciences, Inc. 6645 Nancy Ridge Dr. San Diego, CA 92121 Phone: 800/788-4446; 858/622-0560 E-mail: bugsy@qalife.com
VET-RPLA TD920 [Used to identify V. cholerae enterotoxin]	Reversed passive latex agglutination	24 h (bacterial culture)	Oxoid, Inc. Contact: Jim Bell 217 Colonnade Rd. Nepean, Ontario K2E 7K3 Canada Phone: 613/226-1318 E-mail: jbell@oxoid.ca

[1]Includes enrichment

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