CLINICAL MICROBIOLOGY REVIEWS, July 1988, p. 245–267
0893-8512/88/030245-23$02.00/0
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Vol. 1, No. 3

Current Perspectives on the Epidemiology and Pathogenesis of Clinically Significant *Vibrio* spp.

J. MICHAEL JANDA,* CATHERINE POWERS, RAYMOND G. BRYANT, AND SHARON L. ABBOTT

Microbial Diseases Laboratory, California Department of Health Services, Berkeley, California 94704

HISTORY

The genus *Vibrio* has until recent times been synonymous with disease caused by group O1 *Vibrio cholerae* due to the unique association this species has with epidemic and pandemic cholera (63). Since 1817, seven pandemics attributed to *V. cholerae* O1 have been recorded, with notable outbreaks such as the "Broad Street Pump" episode of London in 1849 describing the epidemiology and mechanism of disease transmission of epidemic cholera. The original description of a vibriolike organism as the etiologic agent of this illness is attributed to Pacini in 1854, although more than 30 years elapsed before the first successful isolation of this bacillus took place (10). Despite these early accomplishments, it has only been during the past 10 years that a greater appreciation for the increasing number of species capable of causing vibrio infections in humans has occurred (135, 198; J. M. Janda and R. G. Bryant, Clin. Microbiol. Newsl. **9**:49–53, 1987).

In the first edition (1970) of the *Manual of Clinical Microbiology* and the eighth edition (1974) of *Bergey's Manual of Determinative Bacteriology*, only two *Vibrio* species were recognized as human pathogens (*V. cholerae* and *V. parahaemolyticus* biotypes 1 and 2 [*V. alginolyticus*])

* Corresponding author.

TABLE 1. Current composition of the genus *Vibrio*

Human pathogens (*n* = 11)	Nonpathogens (*n* = 23)
V. alginolyticus	*V. aestuarianus*[a]
V. cholerae	*V. anguillarum*[a]
V. cincinnatiensis	*V. campbelli*
V. damsela[a]	*V. carchariae*
V. fluvialis	*V. costicola*
V. furnissii[b]	*V. diazotrophicus*
V. hollisae	*V. fischeri*[c]
V. metschnikovii	*V. gazogenes*
V. mimicus	*V. harveyi*
V. parahaemolyticus	*V. logei*[c]
V. vulnificus	*V. marinus*
	V. mediterranei
	V. natriegens
	V. nereis
	V. nigripulchritudo
	V. ordalii
	V. orientalis
	V. pelagius[a]
	V. proteolyticus
	V. psychroerythrus[d]
	V. salmonicida
	V. splendidus
	V. tubiashii

[a] Proposed transfer to the genus *Listonella* gen. nov. (116, 155).
[b] Although isolated from clinical specimens, *V. furnissii* has not been conclusively proven to be a human pathogen.
[c] Proposed transfer to the genus *Photobacterium* (116).
[d] Proposed transfer to the family *Aeromonadaceae* fam. nov. (116, 117).

(49, 171). Principally due to the significant advances made in the area of *Vibrio* taxonomy, this list has now expanded to 11 species that have been isolated from clinical specimens (Table 1). Furthermore, the ability of clinical laboratories to accurately identify and distinguish between phenotypically similar vibrios has led to the association of these newly described agents with particular infectious syndromes such as in the case of *V. vulnificus* bacteremia. Other laboratory studies aimed at understanding the pathogenic mechanisms of virulent *Vibrio* spp. at the molecular level are also in progress. Such investigations should lead to identification of risk factors associated with infection, the prevalence of pathogenic species in environmental samples, and prophylactic and therapeutic measures aimed at the prevention and treatment of *Vibrio* infections. The goal of this article is to provide a current overview of these medically significant bacteria and to highlight some of the advances made in the taxonomy, identification, disease spectrum, epidemiology, and virulence properties of pathogenic species. This review primarily emphasizes case reports and studies conducted in the United States since the review by Blake and colleagues (14) in 1980.

TAXONOMY

Since the original description of the family *Vibrionaceae* by Véron in 1965 (206), which included a number of morphologic and phenotypically similar gram-negative bacteria primarily residing in aquatic habitats, the genus *Vibrio* has held a preeminent position within this group. Although vibrios were originally defined on the basis of biochemical characteristics, recent systematic studies have supported the taxonomic position of most members initially included in this group. The latter investigations have relied heavily on deoxyribonucleic acid/deoxyribonucleic acid relatedness data, guanine/cytosine ratios, amino acid sequence divergence of enzymes such as glutamine synthetase and superoxide dismutase (9), and cellular fatty acid profiles (16, 109). Despite general agreement at the genus level, there has been considerable reorganization at the species rank, in part attributed to the phenotypic similarity of many genetic clusters. Of particular interest is the recent work of MacDonell and colleagues in their analysis of 5S ribosomal ribonucleic acid sequence relatedness of >20 different *Vibrio* species (117). Results from this study show a recent ancestral relationship between the genus *Vibrio* and the family *Enterobacteriaceae*. Within genera, 5S ribosomal ribonucleic acid sequences are so highly conserved from one species to the next that small changes (ca. 1%) in such patterns detected between two different isolates usually indicate the presence of distinct species.

Based upon the results of these and other studies, 34 *Vibrio* species are currently recognized in the genus, a third of which are known to be pathogenic for humans (Table 1). Identification matrices (24), constructed from the results of numerical taxonomy studies (212, 213), essentially support such taxonomic subdivisions and provide reliable biochemical characteristics by which each recognizable species can be identified and distinguished from similar members. However, a couple of exceptions should be noted. Studies by MacDonell and Colwell (116) and associates (155), based on the phylogenetic relationship of members of the family *Vibrionaceae* with 5S ribosomal ribonucleic acid sequence analysis, indicate that several current species in the genus *Vibrio* appear to belong to a new genus, *Listonella* (*V. anguillarum*, *V. damsela*, *V. pelagius*, and *V. aestuarianus*), while others should be transferred to *Photobacterium* (*V. fischeri* and *V. logei*) or the family *Aeromonadaceae* (*V. psychroerythrus*). In addition, numerous studies (23, 212) have identified unnamed *Vibrio* phenons and unclustered strains originating from environmental sources, indicating that more species will eventually be added to the list in Table 1.

VIBRIO DISEASE SPECTRUM

Overview

It is apparent from the volume of published reports on documented illnesses caused by *Vibrio* spp. that the relative number of such infections in the United States is on the increase (46, 73). Specific reasons for such a rise are lacking, but many factors probably contribute to this relative increase. Over the past decade both clinicians and laboratory personnel have gained a heightened awareness for the potential role of *Vibrio* spp. in the differential diagnosis of disease in particular patients. This appreciation has led to the availability of improved biochemical and serologic tests to distinguish phenotypically similar vibrios and to identify important serotypes of *Vibrio* species of major public health significance (e.g., *V. cholerae* O1). Laboratory workers also have the advantage of excellent commercially prepared selective and differential media, such as thiosulfate-citrate-bile salts-sucrose agar (TCBS), which enable the specific isolation of vibrios from contaminated body specimens (e.g., feces). Second, exposure to *Vibrio*-containing environmental sources has grown through increased foreign travel, consumption of raw shellfish, and the use of recreational water facilities by the American public. Finally, because of the progress made in the treatment of malignancies, the relative numbers of immunocompromised individuals in the

TABLE 2. Recent *Vibrio* isolates identified at the Microbial Diseases Laboratory[a]

Species	No. of isolates in given yr								%
	1980	1981	1982	1983	1984	1985	1986	1987	
V. cholerae O1	9	1	0	0	0	1	1	0	9
V. cholerae non-O1	3	8	6	7	8	8	8	10	44
V. parahaemolyticus	1	1	6	12	3	1	3	13	30
V. alginolyticus	0	1	0	1	0	1	0	0	2
V. fluvialis	0	0	0	0	2	0	0	0	2
V. vulnificus	0	1	0	4	3	1	1	2	9
V. mimicus	0	0	0	2	0	0	1	0	2
V. furnissii	0	0	0	0	1	0	0	0	1
V. hollisae	0	0	0	0	0	1	0	0	1

[a] From human sources only.

general population are higher. This enlarging group has an increased susceptibility to infection; thus, for the foreseeable future it appears that, due to several factors, the number of *Vibrio* infections in the United States will continue to escalate.

Vibrio spp. have been isolated from virtually every geographic area in the United States. Although the frequency of such infections is generally higher in coastal states lying along the Atlantic seaboard and Gulf coast, almost all locales will occasionally encounter these marine and estuarine bacteria as potential pathogens. Of 713 *Vibrio* isolates recovered from defined anatomical sites and referred to the Centers for Disease Control (46), 75% belong to one of three species (*V. cholerae*, *V. parahaemolyticus*, or *V. vulnificus*). These vibrios originate from many different sources, although gastrointestinal specimens predominate. At the Microbial Diseases Laboratory, we have identified 132 *Vibrio* isolates representing eight different species over the past 8 years (Table 2). Although the numbers isolated vary somewhat annually, *V. cholerae* non-O1 and *V. parahaemolyticus* are the most common species observed. An interesting facet of *Vibrio* infections is the tissue tropism of certain species (Table 3). Excluding the gastrointestinal tract, three species exhibit a primary tropism for extraintestinal body sites: namely, *V. damsela* (wounds), *V. alginolyticus* (wounds, ear), and *V. vulnificus* (blood, wounds). In addition, two other species (*V. cholerae* non-O1, blood; and *V. mimicus*, ear) display significant secondary tropisms (>15% of total isolates) for anatomical sites outside of the gastrointestinal tract. This information not only is useful from a clinical and diagnostic standpoint, but also has implications regarding the overt pathogenicity and mechanism of transmission of such species.

TABLE 3. Anatomical distribution of *Vibrio* species recovered from humans[a]

Species	Site(s) (%)
V. cholerae O1	Gastrointestinal (97)
V. cholerae non-O1	Gastrointestinal (49), blood (22), ear (13)
V. mimicus	Gastrointestinal (81), ear (15)
V. hollisae	Gastrointestinal (94)
V. damsela	Wounds (100)
V. fluvialis	Gastrointestinal (100)
V. furnissii	Gastrointestinal (94)
V. alginolyticus	Wounds (42), ear (34), respiratory (11)
V. parahaemolyticus	Gastrointestinal (90)
V. vulnificus	Blood (66%), wounds (24)
V. metschnikovii	Blood[b]
V. cincinnatiensis	Blood[b]

[a] Based on recent isolates submitted to the Centers for Disease Control.
[b] Only one clinical isolate of each species has been recovered to date.

Since the overall frequency of *Vibrio* infections in any given institution is relatively low, it is of paramount importance to obtain a good medical history on all patients, since several recognized risk factors will suggest *Vibrio* sp. as the possible etiologic agent of the disease. By far the most common feature observed in individuals presenting with *Vibrio* infections (usually gastroenteritis) is a recent history of consumption of raw seafood, particularly oysters (11). Individuals who identify such a food pattern in their background, irrespective of their presenting symptoms, should be considered likely candidates for having a *Vibrio* infection. Other noteworthy conditions that should be considered potentially indicative of *Vibrio* infections include foreign travel (e.g., Mexico), recent immigration, accidental trauma during contact with seawater- or marine-associated products (e.g., shellfish), or gastroenteritis of choleralike ("rice water stools") nature. Although these situations are not in and of themselves pathognomonic for *Vibrio* spp., they are highly suggestive and help to define a specific population upon which additional diagnostic tests for the presence of vibrios should be performed. In one recent study by Bonner et al. (17) on *Vibrio* infections at their institution over a 10-year period, 87% (20 of 23) of the patients interviewed indicated a recent history of contact with the marine environment or associated products.

V. cholerae. Cholera continues to be a devastating disease of immense global significance, particularly in third-world countries (216), and the recent emergence of *V. cholerae* non-O1 as a significant cause of mortality in Bangladesh (25.8% case fatality rate) is an additional problem of major public health concern (80). The seventh pandemic of cholera since 1800 has progressed since the emergence of the El Tor biotype in Indonesia in 1961, with major peaks of activity being recorded from 1961 to 1966 and subsequently during the early 1970s. In 1978, *V. cholerae* biotype El Tor spread from Asia to eight new countries in Africa, and its importance as a major public health concern cannot be denied. Recent studies from Bangladesh have indicated, however, that the classical biotype of *V. cholerae* has reemerged and is rapidly replacing the previously predominant El Tor biotype in this region of the world (166). Despite these epidemiologic changes, with appropriate recognition and treatment of cholera cases in these areas, the case fatality rate has dropped to <1% of infected individuals, although morbidity continues to run high (216). Vaccines are currently under development, based on the immunogenicity of subunits of the toxin or genetically altered (attenuated) strains of *V. cholerae* (43).

Until 1973, no domestically acquired cases of *V. cholerae* O1 infection had been recorded in the United States in >50 years. In that year, however, an unexplained case of cholera was identified in a man who resided in Port Lavaca, Tex. (Table 4). Since this report (211), 44 additional cases of domestically acquired gastrointestinal infection or colonization due to *V. cholerae* O1 have been documented in the United States (12, 28, 29, 88, 98, 114, 136, 169). Most domestic cases of *V. cholerae* O1 infection present as typical choleralike illness with rice water stools, although milder forms of gastroenteritis have been noted. Common clinical features recorded in these cases include copious bowel movements (10 to >30 per day), dehydration, hypotension, nausea, and abdominal cramps. Fever may or may not be

TABLE 4. Domestically acquired gastrointestinal isolates of *V. cholerae* O1[a]

Yr	No. of cases	Location	No. confirmed by:		No. with clinical status of:		Vehicle (no. of cases)	Reference(s)
			Culture	Serology	Ill	Not ill		
1971	1	Texas	1	0	1	0	Unknown	169, 211
1978	11	Louisiana	11	0	9	3	Crab	12, 169
1980	1	Florida	1[b]	0	1	0	Oysters	136, 169
1981	18	Texas	3	15	17	1	Rice (15), shrimp (1), ditch water (1), unknown (1)	88, 98
1984	1	Maryland	1	0	1		Crab	114
1986	13	Louisiana, Florida	8	5	13	0	Crab, shrimp (12), oysters (1)	28, 29

[a] All culture-confirmed isolates were biotype El Tor, serotype Inaba.
[b] This isolate was nontoxigenic.

present. Case-controlled studies of localized *V. cholerae* O1 outbreaks have indicated that recent ingestion of seafood (raw or partially cooked shrimp and crab) or contact with contaminated water is a significant risk factor associated with infection. All reported cases of domestically acquired *V. cholerae* O1 infections have occurred in individuals residing in Gulf Coast states, with only one exception (114). Although a number of the documented *V. cholerae* O1 infections have been temporally and geographically clustered, only one major outbreak involving 16 workers on an oil rig off the coast of Texas has been described (88). Approximately 56% of all documented *V. cholerae* O1 infections in the United States since 1973 have been culturally confirmed, the remainder being substantiated by demonstration of a vibriocidal antibody response (≥1,280) or evidence of serologic conversion (immunoglobulin G, fourfold rise). Besides documented domestically acquired cases of *V. cholerae* O1 diarrhea, cholera in a New Jersey woman returning from vacation in Cancun, Mexico, has also been reported (27). To date, all domestically acquired cases of cholera which have been culturally confirmed have involved isolates that are serotype Inaba, biotype El Tor. Further laboratory studies on these strains have shown them to be strongly hemolytic (as opposed to those recovered from other areas of the world) and to have a unique bacteriophage susceptibility pattern. The latter two properties have led investigators to speculate concerning the long-term persistence of an endemic *V. cholerae* O1 strain in the waters off the Gulf Coast (169). All U.S. *V. cholerae* O1 isolates have been shown to be toxigenic (by Y1 adrenal cell assay, enzyme-linked immunosorbent assay [ELISA], and gene probe) with the exception of one strain (137) recovered from a 46-year-old Florida woman who developed severe diarrhea subsequent to consuming raw oysters.

In the United States, gastroenteritis due to *V. cholerae* non-O1 clearly predominates over enteric infections caused by *V. cholerae* O1 (137). These former strains, previously designated nonagglutinating vibrios due to their failure to react in polyvalent O1 somatic antisera, have been found to be biochemically and genetically identical to *V. cholerae* O1 and have been included in the epithet, *V. cholerae* non-O1 strains. Diarrhea due to *V. cholerae* non-O1 in the United States is almost invariably sporadic in nature and is only rarely associated with defined outbreaks (137). Symptoms may range from a milder gastroenteritis to a more fulminant diarrhea which resembles cholera produced by *V. cholerae* O1, although most cases are of a less severe nature than those caused by the latter serogroup. Morris et al. (137) summarized the findings on 14 cases (domestic, 9 cases; foreign, 5 cases) of *V. cholerae* non-O1 gastroenteritis submitted to the Centers for Disease Control (CDC) in 1979. Chief complaints included abdominal cramps (93%), fever (71%), and bloody diarrhea (29%). The average duration of symptoms was 6 days. The wider range in presenting symptoms seen with *V. cholerae* non-O1 infections as opposed to O1 infections is probably related to the presence or absence of a number of virulence factors. Most non-O1 isolates lack the cholera toxin gene, although other enterotoxigenic mechanisms may or may not be present. As with O1 infections, diarrhea due to *V. cholerae* non-O1 is strongly associated with the ingestion of raw shellfish (oysters) in domestically acquired cases. In the study by Morris et al. (137), the predominant serotype of *V. cholerae* non-O1 recovered from cases of diarrhea was Smith serotype 17, which accounted for 43% of the total isolates. Findings similar to those described above have been reported in individual case reports (98, 119).

V. cholerae has, on occasion, been recovered from a number of extraintestinal sites in humans, including the ears (196), respiratory and biliary tracts (73, 154), blood (134, 162, 175), cerebrospinal and peritoneal fluids (73, 162), wounds (73, 89), and a number of anatomical tissues including gall bladder and appendix (73). Patients colonized or infected at these sites differ in two major respects from individuals harboring *V. cholerae* in their gastrointestinal tract. Persons found to harbor *V. cholerae* at extraintestinal body sites are much more likely to have underlying diseases, and infection is almost always due to *V. cholerae* non-O1. Common underlying physiologic defects associated with extraintestinal *V. cholerae* non-O1 infections include cirrhosis, malignancy, diabetes, peripheral vascular disease, and abnormalities of the gastrointestinal tract (e.g., gastrectomy). The most common extraintestinal infection produced by *V. cholerae* non-O1 appears to be bacteremia sometimes accompanied by central nervous system involvement. Of six recent adult cases of *V. cholerae* non-O1 bacteremia, all affected individuals suffered from major underlying diseases (17, 38, 39, 134, 162). In five of these six individuals, death was directly attributed to *V. cholerae* non-O1, while in the sixth patient the role of *V. cholerae* non-O1 was thought to be contributory to a fatal case of polymicrobic sepsis. Septicemia due to *V. cholerae* non-O1 has also been described in two newborns (134, 162). Both infants had signs of meningitis (as did one of six adult patients); one of these was culturally confirmed by isolating the organism from cerebrospinal fluid. Neither infant presented with diarrhea immediately preceding the septic episode, and no underlying defect in either newborn was detected at the time of admission. Both infants recovered uneventfully after appropriate antimicrobial therapy. In one newborn, infection was thought to

result from contact of the infant's bottle with live crabs (162).

V. cholerae non-O1 has also been recovered as the etiologic agent of a variety of other extraintestinal infections. Hughes et al. (73) described two cases of *V. cholerae* non-O1 cellulitis in one patient with an oat cell carcinoma and in another with abdominal disorders complicated by a subtotal gastrectomy. In each of these instances a potential source of exposure to vibrios was not identified. Bonner and colleagues (17) identified a similar disease in a 66-year-old diabetic male who lacerated his hand while working on a shell bank. All three of these individuals recovered after appropriate antimicrobial therapy or surgical debridement or both. *V. cholerae* non-O1 has been implicated in a case of cholecystitis in a 28-year-old pregnant female (154), and in infection/colonization of the ear ducts in two young adults (196). Finally, one further case highlights the isolation of rare nontoxigenic *V. cholerae* O1 (by Y1 adrenal cell assay, ELISA, and suckling mouse assay) from a leg ulcer of a 45-year-old male who sustained multiple injuries in a car accident. Although the clinical significance of this isolate could not be definitely determined, its apparent multiplication in the wound over a 16-day period suggests that such nontoxigenic isolates may indeed be capable of producing disease (89).

V. parahaemolyticus. Gastroenteritis is the chief clinical manifestation of infection due to *V. parahaemolyticus*, and the biology of this aquatic bacterium has recently been reviewed by Joseph and collaborators (90). Long ago, an association was recognized in Japan between the consumption of raw shellfish and the subsequent development of *V. parahaemolyticus*-induced diarrhea (198). In the United States, *V. parahaemolyticus* gastroenteritis has been recognized since the first reported cases in Maryland in 1971 due to improperly cooked crabs (133). Subsequently, *V. parahaemolyticus* has been recorded as one of the more common vibrio-induced diarrheas observed in the United States. The diarrhea itself is usually self-limiting and secretory in nature, although a more fulminant dysenteric form has been observed (198). Common symptoms associated with *V. parahaemolyticus* diarrhea include abdominal cramps, nausea, and vomiting. One report from Tanzania (132) suggests that *V. parahaemolyticus* may cause choleralike illnesses. In this study, 7 of 1,591 stool specimens suspected of harboring *V. cholerae* yielded *V. parahaemolyticus*. Six of these patients suffered from significant dehydration. However, only one of these persons had typical rice water stools; stools of three patients were watery in consistency, and the other patients had mucus and blood in their feces, clearly atypical for *V. cholerae* O1. In five women the vehicle of transmission appeared to be raw fish.

With *V. parahaemolyticus* gastroenteritis, there is a strong association between the recovery of an enterotoxigenic isolate from a symptomatic patient and production of a cell-free hemolysin on high salt-mannitol agar containing human erythrocytes (termed Kanagawa phenomenon) (90). Over 95% of such clinical isolates recovered from diarrheic stools have been found to be Kanagawa positive. Conversely, isolates that are Kanagawa negative occur more frequently in the environment (99%) and, when isolated clinically, have been more traditionally associated with the carrier state or are of dubious pathogenic significance (106). The latter concept may require revision since 11 of 12 *V. parahaemolyticus* isolates recently involved in a gastroenteritis outbreak in the Maldives in 1985 were found to be Kanagawa negative by both immunologic and genetic techniques (S. Honda, I. Goto, I. Minematsu, N. Ikeda, N. Asano, M. Ishibashi, Y. Kinoshita, M. Nishibuchi, T. Honda, and T. Miwatani, Letter, Lancet **i**:331–332, 1987).

Of particular note is the recent emergence of gastroenteritis attributed to urea-hydrolyzing strains of *V. parahaemolyticus*, a previously unrecognized biotype in regard to pathogenicity (47). Huq et al. (77) analyzed 11 urease-positive *V. parahaemolyticus* isolates recovered from a diarrheal outbreak in Bangladesh in 1975. All isolates, with one exception, were Kanagawa positive. Since this report, urease-positive *V. parahaemolyticus* have been reported from Singapore (107), Brazil (64), the United States (147), and Malaysia (M. Jegathesan and T. Paramasivan, Letter, J. Diarrhoeal Dis. Res. **3**:162, 1985) and have invariably been Kanagawa positive. Oberhofer and Podgore (147) identified a case of urease-positive *V. parahaemolyticus* gastroenteritis in a 61-year-old male who had previously consumed raw oysters. Symptoms resolved after 9 days without any antimicrobial therapy. In 1984, Nolan and co-workers (146) documented an outbreak (seven cases) of urease-positive *V. parahaemolyticus* in Oregon and Washington during a 3-month period in 1981. Six of these individuals developed gastroenteritis (one had a wound infection) subsequent to consuming raw oysters. Isolates recovered from the feces of four persons possessed identical somatic and capsular antigens. Urease-positive *V. parahaemolyticus* has also been identified as the etiologic agent of severe diarrhea in a New York City resident whose gastroenteritis was characterized by high-grade (105°C) fever and multiple (ca. 30) bowel movements over an 8-h period (J. M. Janda, B. Raucher, R. B. Clark, A. Dixon, and E. J. Bottone, Clin. Microbiol. Newsl. **8**:125–126, 1986). The patient had consumed several clams just prior to his illness. Although diarrhea resolved spontaneously over a 9-day period, he required hospitalization for electrolyte replacement to combat dehydration from profuse diarrhea. Here at the Microbial Diseases Laboratory, approximately 70% of the *V. parahaemolyticus* cultures submitted for identification during the past 8 years have been found to be urease positive.

In rare instances, *V. parahaemolyticus* may be involved in infections outside the gastrointestinal tract. Almost invariably in such situations, individuals present with a history of recent trauma or insult to the infected anatomical site. Tacket and coinvestigators (188) presented a case of *V. parahaemolyticus* panophthalmitis in a 48-year-old man who suffered a foreign-body trauma (corneal laceration) to one eye while working with a dredge that had been in contact with a pond at an oil refinery. Despite immediate medical attention which included topical therapy (gentamicin), a fulminant infection ensued which eventually required enucleation of the eye. Culture of the vitreous fluid from the eye grew a Kanagawa-negative *V. parahaemolyticus* isolate. McMeeking and colleagues (125) have also documented an infection in a 27-year-old male who cut the sole of his foot on a clam shell while at a local beach. Both *V. parahaemolyticus* and *V. vulnificus* were recovered from purulent discharge material from the infected area. Treatment with a cephalosporin brought about resolution of his symptoms. The most intriguing extraintestinal infection caused by *V. parahaemolyticus* is a reported case of pneumonia in a 40-year-old man from Louisiana (S. L. Yu and O. Oy-Yu, Letter, Ann. Intern. Med. **100**:320, 1984). The patient worked as a bailer in a plant that produced oil and fertilizer from fish. He presented for medical attention with chills, fever, cough, and dyspnea. Sputum and blood cultures taken at the time of admission yielded *V. parahaemolyticus*. Upon

epidemiologic investigation, infection was thought to have occurred via droplet inhalation. He was treated intravenously with an aminoglycoside (tobramycin) and was discharged after 10 days of therapy.

V. vulnificus. In 1976, Hollis and others (65) at the CDC identified a phenotypically distinct group of halophilic *Vibrio* isolates that were characterized primarily by their ability to ferment lactose and produce the enzyme β-galactosidase. Termed the "lactose-positive *Vibrio*," 20 of the 38 (53%) original isolates analyzed in this study (65) were recovered from blood, suggesting that they were potentially more virulent when compared with *V. parahaemolyticus* or *V. alginolyticus*. These isolates were subsequently confirmed to represent a new species and were named *V. vulnificus*. Collaborative studies conducted by Blake et al. (13) on the clinical characteristics and epidemiology of these *V. vulnificus* infections identified two major presentations: primary septicemia and wound infections. In the former infection, bacteremia was thought to originate via the gastrointestinal tract (consumption of shellfish) or through introduction of the organism into traumatized epithelial surfaces (wounds, cuts, or lacerations). Persons developing *V. vulnificus* septicemia commonly had preexisting liver disease (67%) and had often consumed raw oysters just prior to their bacteremic episode. On the other hand, people with *V. vulnificus* wound infections lacked underlying hepatic disorders, but did indicate recent exposure to seawater or a history of crabbing and were in a particular age group.

Since these initial reports, the clinical disease spectrum of *V. vulnificus* has become well defined, and the inherent invasive capabilities of this *Vibrio* species have been noted (11, 86). *V. vulnificus* bacteremia, with a reported mortality rate ranging from 40 to 60%, is chiefly associated with the consumption of raw shellfish, particularly oysters (13, 86, 189). Medical conditions predisposing to *V. vulnificus* bacteremia include liver dysfunction and syndromes leading to increased levels of iron in serum, such as chronic cirrhosis, hepatitis, thalassemia major, and hemochromatosis. In a number of individuals without laboratory-confirmed liver disease (e.g., cirrhosis), there is a history of heavy alcohol consumption or abuse. Less frequently, people with underlying malignancies or individuals with gastrectomies have also developed *V. vulnificus* bacteremia (41, 71). Chief symptoms associated with *V. vulnificus* sepsis include fever (94%), chills (91%), and nausea (58%). The mean time for developing symptoms of *V. vulnificus* septicemia after ingestion of raw oysters is approximately 38 h. One notable clinical finding in this disease is the absence of significant diarrhea in a large percentage of patients immediately preceding their septicemic illness. In approximately two-thirds of people with *V. vulnificus* bacteremia, skin lesions appear on either the extremities or the trunk as a direct result of sepsis (13, 189). These dermatologic manifestations may take on a number of different appearances including ecythma gangrenosa-like lesions, vesicles or bullae, necrotic ulcers, cellulitis, and papular or macropapular eruptions (13, 205).

Bonner and colleagues (17) reported eight cases of *V. vulnificus* sepsis, five of which originated from lacerations or punctures to the hand or foot while crabbing or cleaning shrimp or after exposure of a stasis ulcer to seawater. All eight individuals had severe underlying diseases, and the overall mortality rate directly attributed to *V. vulnificus* infection was 50%. Johnston et al. (86) reported on a 73-year-old man with acute myeloblastic leukemia who succumbed to infection with *V. vulnificus* after ingesting over two dozen raw oysters. Oysters recovered from his refrigerator yielded *V. vulnificus*, though of a slightly different biotype. Kelly and McCormick (97) described a fatal case of *V. vulnificus* bacteremia in a 38-year-old male which was accompanied by inflammation of the voluntary muscles (myositis). This patient had no underlying disease or hepatic defect. *V. vulnificus* septicemia concomitant with necrotizing fasciitis has recently been documented in two individuals (an 8-year-old boy and a 78-year-old man). The child apparently contracted the bacterium by rubbing contaminated basin water over an abraded area of his thigh (215). Although a stormy clinical course followed, radical debridement allowed for a positive resolution of his illness. The elderly gentleman had ingested raw oysters, and severe fasciitis of both lower extremities developed in conjunction with bacteremia (84). The patient expired from septic shock approximately 24 h after admission to the hospital. Wongpaitoon and others (214) have documented two cases of sepsis and peritonitis attributed to *V. vulnificus* in men from Thailand with alcoholic cirrhosis. Skin lesions and ascites fluid in both cases were positive for the halophilic vibrio, and each later expired; one died from septic shock, while the other died from massive upper gastrointestinal hemorrhage. One interesting report of *V. vulnificus* bacteremia was described in a 75-year-old man from whom blood, bullous fluid, and stool cultures taken after admission to the medical center were positive for *V. vulnificus* (210). The individual had developed diarrhea 2 days before seeking medical intervention for an acute onset of pain in his forearm. He later became hypotensive and died of cardiac arrest. Lastly, Chin and coworkers (30) have identified a case of *V. vulnificus* bacteremia in a 45-year-old homosexual male with liver disease and acquired immunodeficiency syndrome-related complex. He was treated with ampicillin and gentamicin and later recovered, although he subsequently died a year later from sepsis and peritonitis due to *Escherichia coli*.

V. vulnificus can also be the cause of wound infections most commonly presenting as a cellulitis (13). Persons developing such illnesses are often immunologically competent and acquire disease through mild-to-severe trauma of the infected sites. Recovery in most instances is uneventful unless accompanied by secondary bacteremia (17). Although most people who present with *V. vulnificus* wound infections live in coastal areas, Tacket et al. (187) have highlighted two instances in which people with skin trauma became infected (scalp, hand) through contaminated brackish water: one in a New Mexico creek and the other in a reservoir in Oklahoma. Both individuals recovered after appropriate antimicrobial therapy. Probably the most unusual reported case of wound infection attributed to *V. vulnificus* (199) involves the association of this bacterium with endometritis. The patient, a 32-year-old woman, had engaged in sexual intercourse while swimming in Galveston Bay, and presented to a medical clinic with severe pelvic pain. Endocervical culture revealed *V. vulnificus*. Penicillin and doxycycline therapy were instituted, and the patient became asymptomatic.

Excluding the before-mentioned sites, *V. vulnificus* is rarely encountered from other anatomical sources (including the gastrointestinal tract) and the organism, unlike other halophilic *Vibrio* species, has not clearly been implicated as a cause of diarrheal disease. Reasons for this anomaly are unclear despite the fact that some patients with *V. vulnificus* bacteremia have diarrhea immediately preceding or accompanying their septic crisis. The first evidence directly linking this microorganism with gastroenteritis stems from the survey of Johnston et al. (87) identifying *V. vulnificus* as the probable cause of diarrhea in three individuals. All three

persons were males, had a history of alcohol abuse, routinely consumed antacids or cimetidine, and presented to medical personnel with abdominal cramps, although other clinical symptoms varied. Each had eaten raw oysters during the week immediately preceding their gastrointestinal illness. Except for rehydration therapy (one case), no antibiotics were administered, and diarrhea spontaneously resolved, although in one individual it persisted for 1 month.

V. alginolyticus. *V. alginolyticus* is a bacterium of fairly low pathogenicity for humans. Most clinical isolates are recovered from superficial wounds or the ear. Because many of these sites yield polymicrobic flora upon laboratory analysis, the clinical significance of the isolation of *V. alginolyticus* from such infections cannot be determined (T. A. Cuevas, S. J. Cavalieri, P. M. Christiansen, M. A. Bartelt, and R. B. Clark, Clin. Microbiol. Newsl. **9**:154–156, 1987). However, in several well-documented cases, its role in noninvasive disease cannot be disputed. Opal and Saxon (151) reported on an intracranial infection caused by *V. alginolyticus* in a 20-year-old sailor who received a head injury while diving off the coast of Guam. Cultures from the epidural space and frontal bone tissue revealed *V. alginolyticus* (pure culture). He was treated with chloramphenicol with subsequent remission of his symptomatology. *V. alginolyticus* has also been recovered as the reputed agent of conjunctivitis on two occasions. In one instance, a man who worked as a fish cutter developed conjunctivitis; the discharge from this purulent infection yielded *V. alginolyticus* as the sole bacterium (167). In the other case, a conjunctival culture from an elderly gentleman with conjunctivitis grew the identical organism. Although a definitive source for this infection was not determined, his constant handling of sea shell fragments for use as fertilizer in his garden may have been the vehicle of transmission (112). With regard to enteric disease, *V. alginolyticus* has never been definitively established as a bona fide agent of gastroenteritis, and its isolation from feces is a relatively rare event. One recent report, however, suggests a casual relationship between the isolation of this vibrio from fecal material and diarrhea in an infant and young adult (P. Aggarwal, M. Singh, and S. Kumar, Letter, J. Diarrhoeal Dis. Res. **4**:30, 1986).

V. alginolyticus bacteremia has been reported in the literature on very rare occasions. English and Lindberg (44) described a case of *V. alginolyticus* septicemia in a 37-year-old female who had sustained severe burns while on board a recreational boat. After admission to a burn center, quantitative cultures from her legs and thighs grew *V. alginolyticus* in concentrations ranging up to 10^8 colony-forming units/g of tissue. Blood cultures were also positive for this halophilic vibrio, and the patient's clinical course subsequently declined, with death attributed to the severity of her burns and cardiac arrest. In a second case of *V. alginolyticus* septicemia, a 22-year-old male with acute lymphocytic leukemia died as a result of infection; *V. alginolyticus* was recovered from both blood and soft-tissue specimens (17). Numerous factors which potentially contributed to his enhanced susceptibility to infection included neutropenia, chemotherapy, and splenectomy. Finally, we have recently described a case of fatal bacteremia in a 27-year-old man with osteogenic sarcoma due to *V. alginolyticus* and *Pseudomonas putrefaciens* (82). No definite source for his infection could be identified.

V. mimicus. In 1981, Davis and coinvestigators (39) described a new nonhalophilic *Vibrio* species, designated *V. mimicus*, which appeared to be pathogenic for humans. This group had been formerly classified with atypical *V. cholerae* strains by virtue of its inability to ferment sucrose and its negative Voges-Proskauer reaction. Of 30 clinical isolates studied, 24 (80%) originated from feces, while an additional 13% were isolated from patients with external or internal otitis. Gastrointestinal isolates were recovered from diverse geographic locales including the United States, Guam, Mexico, Bangladesh, and the Philippines. Of 21 strains evaluated in a follow-up study, 17 of 19 fecal isolates were recovered from patients with diarrhea (170). Fourteen of these cases were domestically acquired, and a majority of these individuals (31%) had ingested raw oysters just before their episode of gastroenteritis. Two other isolates were recovered from the ear of a lady with long-term chronic otitis and a boy with bilateral otitis externa. Each had been exposed to seawater immediately preceding the isolation of *V. mimicus*. Ciufecu et al. (31) isolated *V. mimicus* on three separate occasions from persons with gastroenteritis in Romania.

V. fluvialis. During the mid- to late-1970s, a new collection of vibrios belonging to Heiberg group III (based on fermentation of sucrose, arabinose, and mannose) was increasingly isolated from the diarrheic stools of infants, children, and young adults in Bangladesh (75). These vibrios had previously been referred to as group EF-6. Taxonomic studies conducted by Lee et al. (111) concluded that these organisms represented a new species and they were named *V. fluvialis*. Since their recognition, *V. fluvialis*-associated diarrhea has principally been reported from cases of diarrhea in the United States. In one instance, *V. fluvialis* was isolated from the feces of an 81-year-old man from Texas who developed profuse diarrhea and subsequently expired due to electrolyte imbalance and respiratory distress (190). In another report (62), *V. fluvialis* was recovered from the stool of a 1-month-old infant with mild gastroenteritis along with another vibrio, *V. furnissii* (see below). A third case involved a 30-year-old man with ileitis who had *V. fluvialis* and *V. mimicus* simultaneously isolated from his stool (D. Watsky, Clin. Microbiol. Newsl. **5**:111, 1983). He had eaten seafood just 2 days prior to his presenting symptomatology. In each of these cases, the significance of the isolation of *V. fluvialis* is clouded due to incomplete medical histories and the presence of other potential enteropathogens. A more recent report identifying *V. fluvialis* as the etiologic agent causing diarrhea in a 46-year-old man who had consumed raw oysters has been published (J. R. Spellman, C. S. Levy, J. A. Curtin, and C. Ormes, Letter, Ann. Intern. Med. **105**:294–295, 1986). Antimicrobial therapy (doxycycline), in addition to intravenous fluids, was administered because of the severity of his symptoms.

V. furnissii. Originally identified as an aerogenic biogroup of *V. fluvialis*, *V. furnissii* was later found by genetic analysis to represent a new species (21). At present, there is very little clinical information concerning the possible role this microorganism may play in diarrheal disease. In the original taxonomic study (21), two of the four strains of *V. furnissii* analyzed originated from the bowel, and the organism also has been implicated in two outbreaks of food-related gastroenteritis. *V. furnissii* has also been recovered from the feces of an infant with diarrhea (along with *V. fluvialis* and from an asymptomatic 16-year-old male from Singapore (62, 108).

V. damsela. *V. damsela* is a marine bacterium that has been exclusively associated with human wound infections. Morris et al. (138) described the isolation of *V. damsela* from the wounds of six individuals, none of whom had underlying diseases. In four of these persons, lesions were erythematous and indurated and exhibited a purulent discharge. A

history of exposure to brackish or salt water was identified in five of six individuals. Three persons were hospitalized and five required wound debridement for successful resolution of their illness; all patients recovered. Clarridge and Zighelboim-Daum (32) reported the isolation of two hemolytic variants of *V. damsela* from a 61-year-old man whose hand infection had resulted from a small laceration he sustained while cleaning a catfish. From a mild superficial wound infection, the disease evolved into a devastating illness typified by an edematous and necrotizing process of the arm with bulla formation. The patient later died from additional medical complications which included disseminated intravascular coagulation. Coffey et al. (J. A. Coffey, R. L. Harris, M. L. Rutledge, M. W. Bradshaw, and T. W. Williams, Jr., Correspondence, J. Infect. Dis. **153**:800–802, 1986) reported on another case of *V. damsela* cellulitis which rapidly progressed to a fulminant process of the hand and arm requiring debridement, fasciotomy, and eventual amputation to successfully resolve the illness. The precipitating incident appeared to be a slight fish fin puncture to the right middle finger of this diabetic male; *V. damsela* was recovered from the infected tissue during surgery.

V. hollisae. Another halophilic vibrio, first described in 1982 and designated *V. hollisae*, has been infrequently isolated from the gastrointestinal contents of individuals with diarrhea (61). Nine cases of *V. hollisae*-associated diarrhea were recently analyzed by the CDC (138). Universal symptoms included diarrhea and abdominal pain, and in over half of these persons a fever and elevated leukocyte count were noted. All but one of these people were admitted to the hospital as a direct consequence of their presenting symptoms. The source of vibrio infection appeared to be raw oysters or clams in six cases and raw shrimp in one; it was unknown in the other two instances. Only one individual had significant underlying disease (hepatic). At the Microbial Diseases Laboratory, we also have seen a severe case of *V. hollisae* gastroenteritis (watery diarrhea, abdominal pain) in a 42-year-old male who consumed raw oysters, fresh crab, and cooked mussels.

Two cases of *V. hollisae* bacteremia have been documented. One illness occurred in a 35-year-old man from Maryland with underlying cirrhosis who died from numerous medical complications which included concomitant sepsis due to *Cryptococcus* sp. (138). No source for the *V. hollisae* bacteremia could be identified. A second patient, a 65-year-old man, developed *V. hollisae* septicemia after consumption of a freshwater catfish (P. W. Lowry, L. M. McFarland, and H. K. Threefoot, Correspondence, J. Infect. Dis. **154**:730–731, 1986). He was treated with tobramycin, cefamandole, and tetracycline and recovered completely.

V. metschnikovii. Only one reported case of clinically significant infection attributed to *V. metschnikovii* has been reported to date, and it involved an 82-year-old diabetic female (83). The patient presented with abdominal pain and cholecystitis, and a blood culture drawn on admission was positive for *V. metschnikovii*.

V. cincinnatiensis. The most recent addition to the list of vibrios pathogenic for humans comes from the report of Bode and colleagues describing a case of meningitis in a 70-year-old man (15). The patient, who often drank heavily, was disoriented upon admission and had questionable nuchal rigidity. A spinal fluid tap revealed a gram-negative bacillus. Subsequent biochemical and genetic studies indicated that it belonged to a new *Vibrio* species which was subsequently named *V. cincinnatiensis* (20). An identical isolate was recovered from the patient's blood. Treatment included moxalactam for 9 days, after which the patient remained well and healthy. No source for the infection could be identified.

PATHOGENICITY AND VIRULENCE CHARACTERISTICS

Cell-Associated Factors

Surprisingly few studies have been published concerning potential cell-associated virulence factors in pathogenic *Vibrio* species, especially the less frequently encountered members of this group. Investigations have been complicated by the inability of workers to associate the presence of such factors in infecting strains with overt pathogenesis. The common use of repeatedly passaged reference strains in experimental studies further clouds this issue. Finally, many virulence factors, both cell associated and extracellular, may have multiple biologic functions or properties, thereby making the study of such molecules even more difficult.

After direct contact, attachment is a necessary prerequisite to *Vibrio* colonization and infection of such surfaces as the gastrointestinal tract and wounds (Table 5). In *V. cholerae* O1 pathogenesis, it is hypothesized that the bacterial flagellum plays a key role in this process (4). Highly virulent strains of *V. cholerae* O1 are actively motile and attach readily to epithelial cell surfaces in vitro. Nonmotile isogenic mutants or other mutagenized strains are attenuated (4, 8, 56). The role of other attachment structures such as adhesins (45, 92), some of which mediate the agglutination of erythrocytes, is far less clear since these molecules are found in a broad range of *V. cholerae* serotypes originating from diverse sources (18) and fail to show a distinct association with the infectious process (5). In *V. parahaemolyticus*, adherence has been linked to the possession of a flagellum and hemagglutinins in Kanagawa-positive isolates (128, 158); other studies, however, have not shown such a clear correlation (79).

Besides adherence properties, cell surface factors regulate resistance to complement-mediated lysis and other naturally occurring host defenses. Studies by Carruthers and Kabat (25), Tamplin et al. (193), and Johnson and colleagues (85) have indicated that the majority of *V. vulnificus* strains are more resistant to the complement-mediated lysis of human serum than either *V. parahaemolyticus* or *V. cholerae*. The predominance of *V. vulnificus* bacteremia in cirrhotic patients, individuals who often lack normal complement component levels, may be explained by the failure of the bacterium to activate either the classical or alternative pathway against this organism upon systemic invasion (139). One recent interesting finding is the observation by Amako and others (1) of a capsulelike material present on the surface of *V. vulnificus* which appears to be associated with serum resistance and virulence in mice. Subsequent studies by various laboratories have supported these initial observations and have extended them to include resistance to polymorphonuclear leukocytes and macrophages (100, 178, 194, 223).

Extracellular Factors

Of all the reported virulence factors associated with pathogenic *Vibrio* species, only the elaboration of cholera toxin by *V. cholerae* O1 and its correlation with a distinct gastrointestinal syndrome (choleralike illness) have been clearly established (135; Table 5). Evidence supporting such a conclusion is based upon genetic investigations involving the

TABLE 5. Potential virulence factors of pathogenic *Vibrio* species[a]

Factor	Assay(s)	In vitro activity	Target system	Species	Reference(s)
Cell associated					
Flagellum	Motility	Adherence to monolayers	GI tract	*V. cholerae*	4, 5, 8, 56
Adhesins	Hydrophobicity, RBC agglutination	Adherence	GI tract	*V. cholerae*, *V. parahaemolyticus*	18, 45, 79, 92, 158
Serum resistance	Serumcidal activity	Increased growth	Blood	*V. vulnificus*	25, 85, 139, 193, 194
Polysaccharides, acidic	Ruthenium red staining	Antiphagocytic, anticomplementary	Blood	*V. vulnificus*	1, 103, 178, 223
Extracellular					
Enterotoxin CT	RIL, Y1, ID, ELISA, gene probe	FA	GI tract	*V. cholerae* O1, *V. cholerae* non-O1, *V. minicus*	126, 184, 185, 202, 220, 221
Entertoxin LT or ST	Suckling mouse	FA	GI tract	*V. cholerae* non-O1, *V. minicus*, *V. fluvialis*, *V. hollisae*	3, 78, 105
Cytolysin LT	Y1, CHO, RBC, animal models	Cell lysis, FA, tissue destruction	Wounds, GI tract	*V. vulnificus*, *V. fluvialis*, *V. damsela*, *V. cholerae* non-O1	54, 55, 78, 101–103, 115, 122
Cytotoxin LT	CHO	Cell death	GI tract	*V. fluvialis*	145, 161, 209
Cytotoxin Shiga	HeLa	Cell death	GI tract	*V. cholerae* O1, *V. cholerae* non-O1, *V. parahaemolyticus*	148
Hemolysin TDH	Wagatsuma agar, probe, ELISA, Elek	Vascular permeability, cell lysis, FA	GI tract	*V. parahaemolyticus*, *V. hollisae*	66, 129, 143, 219, 222
Proteases					
Collagenase	Enzymatic	Destruction of tissue	Wounds, cutaneous tissues	*V. alginolyticus*, *V. vulnificus*	59, 180
Protease	Enzymatic	Vascular permeability, plasma kallikrein-kinin activator	Cutaneous lesions	*V. vulnificus*	104, 130
Siderophore	Arnow, Csaky	Increased growth, iron acquisition	Blood	*V. vulnificus*	177, 217
Mucinase	Enzymatic	Glycoprotein degradation (mucin)	GI tract	*V. cholerae* O1, *V. cholerae* non-O1, *V. mimicus*, *V. fluvialis*, *V. parahaemolyticus*	81, 150, 168

[a] CHO, Chinese hamster ovary cells; CT, cholera toxin; FA, fluid accumulations; GI, gastrointestinal; ID, immunodiffusion; LT, labile toxin; RIL, rabbit ileal loop; ST, heat-stable toxin; RBC, erythrocytes; TDH, thermostable direct hemolysin; Y1, adrenal cells (mouse).

regulation of cholera toxin, attenuated virulence of nontoxigenic or hypotoxigenic strains of *V. cholerae* O1, epidemiologic and serologic surveys of *V. cholerae* O1-infected persons, and human volunteer studies (26, 33, 113, 126, 182). The molecular biology, genetics, physiology, and pathologic effects of cholera toxin in the gut have been the focus of several recent articles and will not be discussed further in this paper (26, 43, 135). Enterotoxins identical or nearly identical to cholera toxin have been noted in some strains of *V. cholerae* non-O1 and *V. mimicus* by a variety of techniques which include antigenic similarity, biologic activity, and their electrophoretic mobility in gels (184, 185), and cholera toxin gene sequences have been detected in these isolates by hybridization to the labile toxin probe of *E. coli* (144, 145). The clinical significance of such cholera toxin-bearing strains remains to be determined.

Excluding isolates harboring the cholera toxin gene, enterotoxigenic activity has been observed in strains of *V. cholerae* non-O1 (144, 145), *V. mimicus* (144), *V. fluvialis* (115, 144), and *V. hollisae* (105). A number of different biologic functions have been ascribed to one or more of these enterotoxins and include fluid accumulation in animal models, the ability to elicit diarrheal episodes in suckling mice, and elongation of Chinese hamster ovary (CHO) cells (3, 105, 115, 144, 145). Until purified to homogeneity, their clinical relevance and biologic as well as physiologic similarities will remain unresolved.

Cytolysins are thermolabile proteins commonly produced

by a number of pathogenic *Vibrio* species. These extracellular enzymes possess lytic activity against eucaryotic cell lines including erythrocytes and are commonly referred to as "hemolysins" (54, 103, 115). Other notable properties attributed to these reputed virulence factors include vascular permeability in the guinea pig model and fluid accumulation in ileal loops (78, 102, 124). In some species, such as *V. cholerae* O1 (classical, El Tor), two distinct cytolysins with different hemolytic properties have been described (161). A number of these cytolysins have been purified to homogeneity in vitro and partially characterized; however, little is currently known about their potential biologic significance in vivo. Some studies have provided indirect evidence that these toxins may play important roles in the disease pathogenesis of *Vibrio* spp. in vivo. Kreger (101) found a direct correlation between the amount of cytolysin produced by individual isolates of *V. damsela* in vitro and their virulence potential in mice as determined by 50% lethal dose values. A similar investigation conducted by Gray and Kreger (55) focused on the pathologic damage induced in mouse skin by the *V. vulnificus* cytolysin when injected intradermally. The authors postulated that this enzyme (when coupled to other factors) might be responsible for the severe tissue damage seen in human wound infections produced by *V. vulnificus*.

In contrast to cytolysins, cytotoxins have been well described only in a limited number of *Vibrio* species, particularly *V. fluvialis* (115, 209). These molecules are biologically and physiologically distinct from the previously described cytolysins and are characterized principally by their ability to cause cell death without lysis. The *V. fluvialis* cytotoxin is a heat-labile substance which causes elongation of CHO cells and has also been referred to as the "CHO cell-killing factor" (115, 209). No pathologic function has been described for this toxin. Besides *V. fluvialis*, a Shiga-like cytotoxin has been observed in some isolates of *V. cholerae* (O1, non-O1) and *V. parahaemolyticus* (148). This Shiga-like substance was detected only in cell lysates and therefore at present is considered a cell-associated factor only. It has been speculated that this molecule might play a role in bloody or dysenterylike diarrhea occasionally associated with some *Vibrio* infections.

In addition to heat-labile hemolysins (cytolysins), a thermostable direct hemolysin elaborated by *V. parahaemolyticus* and associated with the Kanagawa phenomenon has been identified (143). This product, which has also been detected in *V. hollisae* (143, 222) possesses enterotoxigenic and vascular permeability-like activity in vivo (219), although the former property has been disputed by some researchers (66). Its potential role in foodborne infections due to Kanagawa-positive *V. parahaemolyticus* is still the subject of conjecture.

Many other extracellular factors produced by one or more pathogenic vibrios have been reported, only a limited number of which will be mentioned here. These include proteases active against collagen (59, 180) and elastin (104), those that enhance vascular permeability in guinea pigs (130), mucinases (81, 150, 168), and siderophores (177, 217). Although the role of such enzymes in virulence is undefined, their proposed function(s) is listed in Table 5.

Plasmids

Plasmid carriage among the *Vibrionaceae* of medical importance appears to be sporadic, and, although high-molecular-weight, multiple-antibiotic resistance plasmids occur in *V. cholerae* and *V. parahaemolyticus*, most plasmids detected are of low molecular weight and are cryptic (Y. Mitoma, T. Aoki, and J. H. Crosa, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, H84, p. 119). Attempts to correlate extrachromosomal elements with phenotypic or virulence characteristics generally have been unsuccessful (38, 141, 203), although *V. anguillarum* carries a plasmid, pJM1, that encodes an iron-sequestering system (37). Hamood et al. (58) have shown that the R factor pVH2 of *V. cholerae* enhances virulence probably by regulation of virulence-related genes, since its effect was not related to cholera toxin, hemolysin, or colonization factors. Conversely, other plasmids, most notably sex factor P of *V. cholerae*, cause a decrease in pathogenicity by reducing cholera toxin production (57, 141).

R factors in *Vibrio cholerae* O1 serovars are infrequent, usually disappear spontaneously, and belong predominantly to incompatibility group C, although they are of diverse origin and confer different antibiotic resistance markers (52, 60). Glass et al. (51) proposed that the lack of antibiotic pressure eliminates the selective advantage of strains carrying R factors. However, the virtual absence of other plasmids in *V. cholerae* O1 serovars of either environmental or clinical origin versus non-O1 *V. cholerae* (53, 141) points to multiple factors being responsible for the paucity of plasmids in *V. cholerae* O1 serovars. This observation, however, may be restricted to El Tor strains (neither Newland et al. [141] nor Glassman et al. [53] indicated the biotypes of their O1 serovars, but all strains were isolated during the seventh pandemic), because Cook et al. (36) found that, while their El Tor strains lacked plasmids, classical strains typically contained both a 21- and a 3-megadalton plasmid. Classical strains tested were isolated from both the sixth and seventh pandemic, and *Hin*dIII restriction digests of their plasmids were identical, although no function could be assigned to these genetic elements.

The molecular basis for exclusion and instability of most plasmids in *V. cholerae* is unknown, and the apparent lack of plasmids may be complicated by difficulties encountered in isolating extrachromosomal material from vibrios (2, 36, 38, 141). Sometimes pooling of as many as four plasmid preparations is required before bands are visible. Davidson and Oliver (38) found it necessary to eliminate lysozyme from the procedure to prevent premature cell lysis during which extrachromosomal restriction endonucleases and deoxyribonucleases are released.

Animal Models

In experimental studies, investigators have used a variety of animal models to assess the capabilities of different *Vibrio* spp. to elaborate a heat-labile or a heat-stable enterotoxin. These systems include the ligated intestinal loop (ileal loop, De test), infantile rabbit test (Dutta test), and suckling mouse assays (76, 78). Even when cell-free supernatants have failed to produce detectable enterotoxin-like activity, the ability of live cultures to produce fluid accumulation in rabbit ileal loops has been demonstrated with such organisms as *V. cholerae* non-O1 (120). In addition to intestinal models, Simpson et al. (176) have recently described a mouse model for studying wound infections caused by *V. cholerae* O1 and non-O1. Infected animals were scored for formation of necrotic lesions or fatal infections or both subsequent to subcutaneous inoculation with 10^7 cells of individual strains.

Virulence Markers

Since few cell-associated or extracellular virulence factors have been definitively established to play a role in *Vibrio*

TABLE 6. Incidence of vibrios in the environment, recent surveys (United States)[a]

Area sampled	Survey date(s)	*V. cholerae*	*V. parahaemolyticus*	*V. vulnificus*	Reference
Chesapeake Bay	June 1979–Aug. 1981	Water: 0.7–46.0 MPN/liter Plankton: >50% positive Oyster harvest sites: 0.3–0.9 MPN/liter (4 of 9 samples negative)	Water: 0.3–300 MPN/liter Sediment: 0.3–300 MPN/g Oysters from 18 harvest bars: all samples positive Plankton: 10^7–10^8 g	Rarely isolated Highest concn: Water: 230 MPN/liter Sediment: 9.0 MPN/cm^3 Oysters: 2.3 MPN/g	35
Florida	Apr. 1980–Aug. 1981	Shellfish: mean 4.1 MPN/g 1% of isolates were type O1			70
Louisiana	Jan. 1980–July 1981	Non-O1 range: <3.0–>110,000 MPN per (liter water or g of sediment	Water: <3.0–2,100 MPN/liter	Water: <3.0–10,000 MPN/liter	6
Northeastern coast	Apr.–Nov. 1983, 1984, 1985		Routinely isolated July to Sept. each yr	Water: <1–4,000/100 ml, direct count Shellfish: 84 of 96 samples positive	197
Santa Cruz, Calif.	Jan.–Mar. 1983	Water, non-O1: postiive in 5 of 8 4-liter samples 0.04–4.6 MPN/liter			99
West coast (Wash., Ore., Calif.)	June–Oct. 1984	Non-O1 found in 23 estuaries and 44.6% of 529 water, sediment, and shellfish samples		Found in 5.9% of 529 water, sediment, and shellfish samples	95

[a] MPN, Most probable number.

pathogenesis, specific markers to identify pathogenic strains of a given species are generally lacking. Excluding the association of serogroup O1 of *V. cholerae* with cholera toxin production, only the elaboration of the thermostable direct hemolysin by enterotoxigenic strains of *V. parahaemolyticus* has been clearly demonstrated. This hemolysin, detected on Wagatsuma agar which contains human O erythrocytes, is preferentially found associated with strains causing gastrointestinal disease as opposed to isolates recovered from environmental sources. Recovery of strains producing such a factor, termed Kanagawa positive, from the fecal contents of symptomatic individuals usually indicates a causal relationship with the disease.

One interesting finding of potential clinical significance is the association of *V. vulnificus* colonial morphology with mouse virulence. Opaque *V. vulnificus* colonies grown on nutrient or heart infusion agar are pathogenic for mice and guinea pigs, whereas translucent colonies are not (178, 223). The opaque colony has been further shown to be associated with an external ruthenium red-staining polysaccharide capsular material present on the cell surface.

ENVIRONMENTAL STUDIES

Ecology

The vibrios are aquatic bacteria found in a wide variety of environmental water sources including the ocean, estuaries, lakes, and ponds. Extensive work has been done on the natural incidence and survivability of vibrios in the aquatic environment (34, 68, 74, 93, 191, 197), and the picture that emerges is a fascinating one of seasonal growth and decline coupled to special ecologic relationships with higher copepods and other plankton. The vibrios may also be required for a balanced ecosystem in the marine environment, and their association with higher organisms may provide a beneficial effect on salt retention by copepods and other organisms which play vital roles in the food chain (35). When present in any water habitat, vibrios may constitute a major part of the aquatic bacterial flora. Table 6 gives a summary of recent surveys on the incidence of pathogenic *Vibrio* spp. in their natural habitat, although some of the studies have focused only on *V. cholerae*. In general, vibrios have been shown to reach high concentrations in the marine waters along the East and Gulf coasts, especially during the summer months; pathogenic vibrios are also present in the cooler waters off the West coast, but in much lower concentrations.

The most important modulators of environmental concentrations of vibrios worldwide are water temperature and salinity (96, 179). The concentration of vibrios varies directly with temperature, with higher numbers occurring in waters from 17 to 35°C containing 5 to 25‰ salinity. Individual *Vibrio* species, however, have different optimum requirements, and although *V. cholerae* does not require added salt when growing on laboratory media, it actually prefers 2 to 20‰ salinity and temperatures between 20 and 35°C in its natural habitat. Physical and chemical attributes of the environment may also modulate toxin production by *V. cholerae* (192). Halophilic vibrios, on the other hand, require Na^+ for growth and can reach very high concentrations in waters of 5 to 8‰ salinity. In any aquatic source, the *Vibrio* concentration may vary over relatively short periods of time according to the amount of local rainfall and amount of freshwater runoff.

Epidemiology

It has become apparent that large coastal and inland areas of the United States have become endemic foci for pathogenic *Vibrio* spp. Although most vibrio infections are reported from coastal areas, recent reports of gastrointestinal and wound infections in animals and humans have also been documented from inland areas far from any sea coast (159, 187). Most of these infections have involved *V. cholerae* non-O1 and *V. vulnificus*.

Consumers of raw, poorly cooked, or recontaminated shellfish are at risk for vibrio infections. All types of shellfish products may be involved including oysters, mussels, clams, shrimp, and crabs, all of which are available almost universally throughout the United States. The risk of infection with *Vibrio* spp. is highest with filter-feeding bivalves since they concentrate contaminants in nature from the surrounding water.

Contact with contaminated water is a risk factor mainly for wound infections and may involve the direct immersion or splashing of water in or onto open wounds or mucous membranes or entry through fish hook wounds, as well as less obvious routes such as cuts by broken shells or shark and other fish bites. Infective doses for *Vibrio* spp. and their relative incidence in clinical specimens and the environment have been previously summarized (Janda and Bryant, Clin. Microbiol. Newsl. **9**:49–53, 1987).

Most pathogenic vibrios isolated from the environment exhibit wide strain-to-strain variation in virulence. Isolates of *V. parahaemolyticus* from clinical infections are almost always Kanagawa positive (hemolytic for human erythrocytes), while isolates from the environment, including actual seafoods directly implicated in outbreaks, are nearly always Kanagawa negative and nonvirulent. The variation in virulence of *V. cholerae* non-O1 isolates or host resistance factors potentially allows some strains to cause only mild to severe gastroenteritis, while others may be involved in bloodstream invasion or deeper body sites. Unlike other halophilic vibrios, the virulence of environmental strains of *V. vulnificus* appears to be indistinguishable from that of clinical isolates (200, 201). Although some strains may produce both a virulence-associated opaque colony type and an apparently nonvirulent translucent colony type, most environmental strains possess the same complement of cytotoxins, cytolysins, and other phenotypic characteristics as clinical isolates.

Currently, the most serious extraintestinal vibrio infections are caused by *V. vulnificus* in immunocompromised patients. Most of these cases have been linked to the consumption of raw oysters from the East and Gulf coasts. Infection and mortality with *V. vulnificus* bacteremia is related to the immune status of the host, and, as with other halophilic species of *Vibrio*, little is known about the infective dose of these newly recognized groups for either compromised or uncompromised individuals.

Isolation from Environmental Sources

The isolation of pathogenic vibrios from environmental sources as part of an outbreak investigation or surveillance activity requires planning to be successful. A different plan of attack is needed for each type of sample. Samples to be examined include water, sewage, sediment, plankton, fish, and shellfish. Maximum sensitivity is realized by processing samples as soon as possible after collection. Overall microbial changes during unavoidable storage periods are minimized at cold temperatures, but the effect of cold stress on the vibrios may become harmful, especially when combined with other factors.

Since the concentration of vibrios may be low in water and sewage samples, some type of concentration procedure is generally required. Filtration through gauze or a membrane filter, or use of a Moore swab, has been used successfully by various investigators (7, 183). Columns of polystyrene beads coated with antibodies to *V. cholerae* O1 have been used to concentrate these organisms from water samples and to separate them from the predominant non-O1 types (72). Shellfish samples not examined immediately should be held refrigerated as living shellstock. Homogenization of oyster meats may release lethal factors for pathogenic vibrios that are very detrimental when combined with the stress of cold storage for any length of time. This effect has been studied in some detail in *V. vulnificus* (149). While moderate to high concentrations of vibrios may be successfully detected and enumerated directly, many samples will require enrichment prior to plating. This is especially true for *V. cholerae* O1, which tends to be present in very low concentrations. Direct isolation techniques have been most successful with *V. parahaemolyticus* and *V. vulnificus* (22, 94). Enrichment broth media used for pathogenic vibrios capitalize on the ability of these organisms to grow rapidly at an alkaline pH (8.5 to 9.0), resist the inhibitory effects of bile salts and sodium tellurite, and tolerate salt. The most commonly used enrichment medium for all vibrios of medical interest is alkaline peptone water. Salt colistin broth, glucose salt teepol broth, and arabinose-ethyl violet broth have been recommended for enrichment of *V. parahaemolyticus* but not for other species; alkaline bile peptone water has been recommended for *V. cholerae* but has not been evaluated for other species (40, 67, 69, 93, 183, 204). Incubation times of 6 to 18 h at 35 to 37°C have commonly been used for enrichment (6 h, optimum), although a new procedure designed to recover *V. cholerae* involves incubation in alkaline peptone water at 42°C for 18 h (40).

A variety of different protocols for the isolation of pathogenic vibrios from environmental samples has become popular in recent years. Overall, TCBS agar is most commonly used. A combination of alkaline pH and bile salts gives the medium selectivity, while sucrose fermentation is the differential ingredient. A medium designed for isolation of *V. cholerae* is taurocholate-tellurite gelatin agar. Tellurite is reduced to nontoxic tellurium by *V. cholerae* on this medium, and it is possible to do an oxidase test directly from the plate. A new medium, which highlights alkyl sulfatase production by *V. cholerae* and *V. vulnificus*, is sodium dodecyl sulfate-polymyxin B-sucrose medium. This medium has been evaluated for the direct enumeration of *V. vulnificus* from shellfish (22). The biochemical screening of moderate to large numbers of presumptive vibrio isolates from environmental samples presents a challenge for the cost-effective use of laboratory time. Several schemes are useful depending on the isolation method used and the targeted organisms. One common procedure tests for oxidase production, salt requirement for growth, susceptibility to vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine), gelatinase production, and β-galactosidase activity.

For *V. cholerae*, a multitest medium (VC medium) has been devised that screens for several reactions in a single tube (140). The medium is similar in principle to triple sugar iron agar and tests for fermentation of glucose and inositol or arabinose, arginine dihydrolase, indole production, H_2S production from sodium thiosulfate, and production of gas.

This medium has been of value in large environmental surveys for *V. cholerae*. Regardless of the initial screening steps, the key biochemical evaluations usually involve tests for lysine decarboxylase and arginine dihydrolase, sucrose/salicin/arabinose fermentation, *o*-nitrophenyl-β-D-galactopyranoside reaction, and indole production. Isolates can then be grouped if not identified based on any one of several systems. Again, the order of the various screening tests and further evaluation are generally customized to the particular study and target organisms. Final identification may require additional tests and the services of a reference laboratory.

Various virulence assays may be used to characterize potentially pathogenic isolates such as the Kanagawa test for *V. parahaemolyticus* and Y1 adrenal cell assays for cholera and choleralike toxins, as well as other assays for production of cytotoxins, cytolysins, and other factors. Mouse lethality assays may be performed with normal or iron-supplemented mice. Serological and phage typing of isolates may be required for epidemiological purposes.

Public Health Aspects

The protection of the public from vibrio infections theoretically involves control of both foodborne exposure and contact with contaminated waters. In practice, this goal is extremely complicated and elusive. There are approximately 10 million acres of approved shellfish-harvesting areas in the United States. Governmental agencies monitor recreational water sources as well as shellfish and harvesting locales primarily for indicator organisms and total heterotrophic counts. These studies provide no useful information on the actual incidence and concentration of pathogenic vibrios except in the case of fecally polluted products. Recent work by Xu et al. (218) on nonculturable *E. coli* and *V. cholerae* in aquatic environments raises further questions about the efficiency of standard monitoring techniques, since certain pathogens appear to exist in a viable, but nonrecoverable, state. Indicator concepts may have to be reviewed and possibly modified in light of these findings. Limits on the vibrio content of fish and shellfish have not generally been applied; however, a recommendation from the International Commission on Microbiological Specification for Foods has set the upper limit for *V. parahaemolyticus* in raw shrimp at 100 colony-forming units/g.

Survival of Pathogenic Vibrios in Stored Shellfish

Various studies have determined the effects of storage on the concentration of vibrios in oysters and clams, both before and after shucking (67, 69). Storage of clams at the usual cold temperatures appears to have little effect on the generally low levels of vibrios present. Storage of oysters, however, results in some interesting changes in different *Vibrio* concentrations. In living shellstock at the coldest storage temperature (2°C) used, the concentrations of *V. cholerae* may increase 1 log after 7 days, while those of *V. parahaemolyticus* and *V. vulnificus* decline. At the most commonly used storage temperatures of 4 and 8°C, only *V. vulnificus* shows an increase (up to 2 logs at 8°C); at nonrefrigeration temperature (20°C), both *V. parahaemolyticus* and *V. vulnificus* show larger increases. The total bacterial count and coliform most probable number may correlate with vibrio concentrations after 7 days of storage.

The control of harmful contaminants in shellfish, including pathogenic *Vibrio* spp., by the cleansing processes of relaying or depuration is being carried out by a number of shellfish producers on both sea coasts. This type of cleansing lowers the concentrations of most bacteria but may have little effect on viruses. If the level of contamination is very high, relaying and depuration may not work at all. One control measure that might be successfully applied is the strict regulation of the type of shellfish products shipped beyond local markets. Pathogenic vibrios may reach peak concentrations after 7 days of storage as living shellstock, but tend to decline in processed meats. Heat processing is another possible solution. A pasteurization routine (57.2°C, 30 min) for packed oysters has been proposed (19).

LABORATORY IDENTIFICATION

The laboratory identification procedures discussed will be limited to the *Vibrio* spp. which have been implicated as a cause of human disease or have been isolated from clinical specimens (Table 1).

Members of the genus *Vibrio* are fermentative, facultative-anaerobic, gram-negative, straight or curved motile rods (with a single polar flagellum in liquid media) whose growth is stimulated by Na^+ which, except for *V. cholerae* and *V. mimicus*, is an absolute requirement (10). They are anaerogenic (exception, *V. furnissii* and some strains of *V. damsela*) and, except for *V. metschnikovii*, oxidase positive, and they reduce nitrate to nitrite. Most are susceptible to the O/129 vibriostatic compound (10).

Certain key characteristics that aid in the separation of *Vibrio* spp. from other medically significant bacteria with which they may occasionally be confused (i.e., *Enterobacteriaceae*, *Pseudomonas*, *Aeromonas*, and *Plesiomonas* spp.) are the production of oxidase, fermentative metabolism, requirement of NaCl for growth, and susceptibility to O/129. The positive oxidase reaction will differentiate them from members of the *Enterobacteriaceae*, which are oxidase negative. Their fermentative metabolism distinguishes them from the oxidase-positive *Pseudomonas* spp., which are oxidative. The oxidase-positive *Aeromonas* and *Plesiomonas* spp. do not require NaCl for growth. *Aeromonas* spp. are resistant to O/129 and *Plesiomonas* strains vary in their susceptibility to this compound.

The techniques and media available routinely in most clinical laboratories can be used to isolate the potentially pathogenic *Vibrio* spp. However, the recognition that an isolate is a vibrio and its identification to species may be more difficult. Of the *Vibrio* spp. that have been isolated from extraintestinal sites, *V. cholerae* non-O1, *V. vulnificus*, and *V. alginolyticus* have been the most frequently recovered species.

Specimen Collection and Transport

Specimens from extraintestinal pathological material such as wound, blood, and other body fluids and sites should be collected and processed by routine procedures. The 0.5% NaCl present in the blood and nutrient agars used commonly in most laboratories will support growth of the clinically significant vibrios. When clinical history indicates that there has been exposure to seawater or seafood, TCBS agar can be included.

Stool specimens should be collected early in the disease before antibiotic therapy has begun and inoculated onto isolation media as soon as possible after collection. For organism recovery, rectal swabs are satisfactory in the acute stage of the disease, but are not reliable from contacts, convalescents, or patients on appropriate antimicrobial ther-

apy as the numbers of vibrios may be few. If there is a delay or if specimens must be transported, they should be placed in Cary-Blair transport medium. Buffered glycerol saline is unsatisfactory because glycerol is toxic to vibrios. Direct microscopic examination of stool specimens is not recommended since it may not be possible to distinguish *Vibrio* sp. from other motile, straight, or curved rod-shaped bacteria (198).

Selective Media

Of the many selective media developed for the isolation of vibrios, TCBS agar is the most convenient and widely used, highly selective medium (157). It is suitable for most of the enteropathogenic vibrios; however, *V. hollisae* may grow poorly or not at all. It is available commercially and easy to prepare, and it differentiates sucrose-fermenting (yellow) from non-sucrose-fermenting (blue-green) colonies; other organisms are almost completely inhibited. Some of the problems in recovery of vibrios on TCBS may be due to variation of the medium. Some brands may be very inhibitory and unsuitable. There also may be variations between different lots by the same manufacturer or different batches of the same lot (123, 142). Commercially available dehydrated TCBS agar should be prepared carefully and not overheated; quality control of each lot and batch is extremely important (110, 195, 213).

Pathogenic *Vibrio* spp. usually grow on MacConkey agar and will be colorless except for the lactose-fermenting *V. vulnificus*. They generally do not grow on the other enteric-selective plating media, and the sucrose-fermenting strains, if they do grow, may not be detected on the sucrose-containing xylose-lysine-deoxycholate and Hektoen enteric agars.

Enrichment Media

Enrichment broth is recommended for isolation of vibrios from convalescent and treated patients. Alkaline peptone water with 1% NaCl (pH 8.5) can be used for isolation of the human vibrios since they grow very rapidly at alkaline pH compared with normal fecal flora. A volume of 20 ml should be used for isolation of vibrios from feces because smaller volumes do not remain adequately alkaline. The broth should be subcultured to TCBS after 5 to 6 h at 35°C; longer incubation allows for overgrowth of vibrios by other organisms. Longer incubation (18 to 20 h) can be used if the broth is incubated at lower temperatures (18 to 22°C) (110).

Gram Stain

All vibrios are short, gram-negative, straight or curved rods. Curvature is not diagnostic, since it is not always obvious in Gram stains, and other organisms may also show curvature. The microscopic appearance is influenced by the medium on which the organism is grown. Many vibrios are highly pleomorphic and may show involuted forms, particularly when growth conditions are suboptimal.

Biochemical Characteristics

Media commonly used for identification of members of the *Enterobacteriaceae* can be used if the NaCl concentration is increased to 1% (wt/vol). Halophilic vibrios will not grow, or will grow poorly, in Voges-Proskauer, nitrate, 1% peptone, and Moeller decarboxylase broths without added salt. Growth of these species may occur in other media because of the minimal amounts (usually 0.5%) of salt present, but biochemical reactions may not be reliable. The oxidase test must be performed on growth from media containing no fermentable carbohydrates. Susceptibility to the vibriostatic agent should be performed on media low in salt. It has been shown by Merkel (127) that both NaCl and $MgCl_2$ block the vibriostatic action of O/129.

TABLE 7. Biovars of *V. cholerae* O1[a]

Reaction	Response by biovar:	
	Classical	El Tor
Hemolysis of sheep erythrocytes	−	+
Voges-Proskauer	−	+
Chicken erythrocyte agglutination	−	+
Polymyxin B, 50 IU	S	R
Classical phage IV	S	R
El Tor phage 5	R	S

[a] S, Susceptible; R, resistant.

The methods used for identification of the clinically significant *Vibrio* spp. have been described in detail by Farmer et al. (46) and Furniss et al. (48); therefore, only some of the differential characteristics of particular significance will be covered here.

The lysine and ornithine decarboxylase and arginine dihydrolase activities of the pathogenic vibrios are useful for separating them into groups. *V. cholerae*, *V. mimicus*, *V. parahaemolyticus*, *V. vulnificus*, *V. cincinnatiensis*, and *V. alginolyticus* are lysine decarboxylase positive and arginine dihydrolase negative. *V. fluvialis*, *V. furnissii*, and *V. damsela* are arginine dihydrolase positive. *V. hollisae* is negative for lysine and ornithine decarboxylases and arginine dihydrolase.

The lack of oxidase and nitrate activity distinguishes *V. metschnikovii* from the other pathogenic species. *V. cholerae* and *V. mimicus* are characterized by their ability to grow in nutrient broth without added NaCl. These two biochemically similar species can be differentiated by sucrose fermentation; *V. cholerae* is positive, while *V. mimicus* is negative. The classical and El Tor biovars of *V. cholerae* O1 can be distinguished by the Voges-Proskauer reaction, hemolysis of sheep erythrocytes, susceptibility to polymyxin B, agglutination of chicken erythrocytes, and lysis by bacteriophage (Table 7).

Production of gas from carbohydrates by *V. furnissii* (previously designated as an aerogenic biovar of *V. fluvialis*) will distinguish it from *V. fluvialis*. *Aeromonas* sp., frequently confused with *V. fluvialis*, will grow in nutrient broth without NaCl, while *V. fluvialis* will not.

Many of the *V. parahaemolyticus* strains isolated in recent years have been urease positive. Of the 38 strains tested by the Microbial Diseases Laboratory since 1982, 25 are urease positive. Most strains of *V. parahaemolyticus* isolated from humans are Kanagawa positive, while those from the environment are usually negative (90). The Kanagawa test is based on the detection of a heat-stable hemolysin in a special medium (Wagatsuma agar) which contains human erythrocytes (129).

The rapid fermentation of lactose and salicin aids in the recognition of *V. vulnificus*. Additional reactions in selected tests that differentiate 11 *Vibrio* spp. as well as *Aeromonas* and *Plesiomonas* spp. are given in Table 8.

TABLE 8. Tests for differentiation of members of the *Vibrionaceae* from humans[a]

Test	*V. cholerae* O1 and non-O1	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*	*V. alginolyticus*	*V. cincinnatiensis*	*V. fluvialis*	*V. furnissii*	*V. damsela*	*V. hollisae*	*V. metschnikovii*	*Aeromonas* spp.	*Plesiomonas* spp.
Oxidase	+	+	+	+	+	+	+	+	+	+	−	+	+
NO_3-NO_2 + 1% NaCl	+	+	+	+	+	+	+	+	+	+	−	+	+
Indole + 1% NaCl	+	+	+	+	+	−	−	−	−	+/−	+/−	+/−	+
Voges-Proskauer + 1% NaCl	+/−	−	−	−	+/−	+	−	−	+	−	+/−	+/−	−
Urease	−	−	−/+	−	−	−	−	−	+	−	−	−	−
Lysine decarboxylase + 1% NaCl	+	+	+	+	+	+	−	−	+/−	−	−/+	+	
Ornithine decarboxylase + 1% NaCl	+	+	+	+/−	+/−	−	−	−	−	−	−	−	+
Arginine dihydrolase + 1% NaCl	−	−	−	−	−	−	+	+	+	−	+/−	+/−	+
Fermentation of													
Sucrose	+	−	−	−/+	+	+	+	+	−	−	+	+/−	−
Lactose	(+)/−	+/−	−	+	−	−	−	−	−	−	+/−	−/+	+/−
L-Arabinose	−	−	+	−	−	+	+	+	−	+	−	+/−	−
Gas from glucose	−	−	−	−	−	−	−	+	−/+	−	−	−/+	−
Growth in nutrient broth													
0% NaCl	+	+	−	−	−	−	−	−	−	−	−	+	+
3% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+
6% NaCl	+/−	+/−	+	+/−	+	+	+/−	+/−	+	+/−	+	−	−
8% NaCl	−	−	+	−	+	−	−	−	−	−	+/−	−	−
10% NaCl	−	−	−	−	+/−	−	−	−	−	−	−	−	−
Susceptibility to O/129													
10 μg	S	S	R	S	R	R	R	R	S	R	S	R	R/S
150 μg	S	S	S	S	S	S	S	S	S	S	S	R	S
Growth on TCBS	Y	G	G	G/Y	Y	Y	Y	Y	G	G/−	Y	−	−

[a] +, Most strains positive; −, most strains negative; +/− or −/+, variable reaction (predominant reaction shown as the numerator); ( ) = delayed reaction; S, susceptible; R, resistant; Y, yellow colonies; G, green colonies.

Rapid or Semiautomated Methods for Identification

Commercial kits used by many laboratories have improved and usually identify the more common *Vibrio* spp., but there are still problems with some of the less common halophilic species (152, 153). Unreliable results may occur because the halophilic vibrios may grow poorly, even when 0.85% NaCl is substituted for the usual suspending medium (46). MacDonnell et al. (118) found that *Vibrio* spp. gave more positive results if artificial seawater was used as a suspending medium. At present, the data bases of these kits are not adequate to identify all of the less common species, and misidentification is common (32, 207).

Serology

All strains of *V. cholerae*, both O1 and non-O1, share a common flagellar (H) antigen. In 1935, Gardner and Venkatraman (50) divided the cholera vibrios into six subgroups on the basis of their somatic (O) antigens. The cholera strains were assigned to O-subgroup 1, and the noncholeragenic vibrios were placed in groups 2 to 6. Today, two typing systems are commonly used for typing *V. cholerae*: that of Sakazaki and colleagues (165, 172) and that of Smith (181). Both place those strains which cause cholera in O group 1. The Smith (181) typing scheme will identify over 72 different types of non-O1 *V. cholerae*, and 83 O-antigenic groups have been recognized by Sakazaki and Donovan (163).

Polyvalent O1 antiserum which will distinguish between O1 and non-O1 *V. cholerae* is available commercially. These sera are usually satisfactory; but in a report by Donovan and Furniss (42), two laboratories using commercial antisera misidentified non-O1 *V. cholerae* as O1. We are also aware of one laboratory in California that obtained agglutination by a non-O1 strain with commercial O1 serum. Subtyping of *V. cholerae* O1, using absorbed antisera, is usually performed in reference laboratories. Two serotypes, Ogawa and Inaba, have been recognized. Hikojima, thought to be an intermediate form, apparently has been confirmed as a specific serotype by Sugiyama et al. (186). They successfully prepared monoclonal antibodies against antigen fractions a, b, and c of *V. cholerae* O1 and developed a monoclonal antibody-sensitized latex agglutination test to subdivide it into three serotypes: Ogawa, Inaba, and Hikojima.

V. parahaemolyticus can be serotyped on the basis of its O and K antigens (90, 164). Commercial antisera for the O and K antigens in this typing schema are available from Nichimen Co., Inc., New York, N.Y. (46). Serotyping is usually limited to strains from outbreaks or to special studies and should be performed in a reference laboratory. Except for studies of Shimada and Sakazaki (173, 174) on *V. fluvialis* and *V. vulnificus*, little is known about the antigenic structure of other species of *Vibrio*.

Antibody Response of the Patient

A retrospective diagnosis of cholera can be established by serologic means provided that the patient has not been immunized. Immune response to *V. cholerae* infection can be demonstrated by the detection of antibodies induced by somatic antigens, using agglutination or vibriocidal antibody assays, and by the detection of antibody (antitoxin) directed

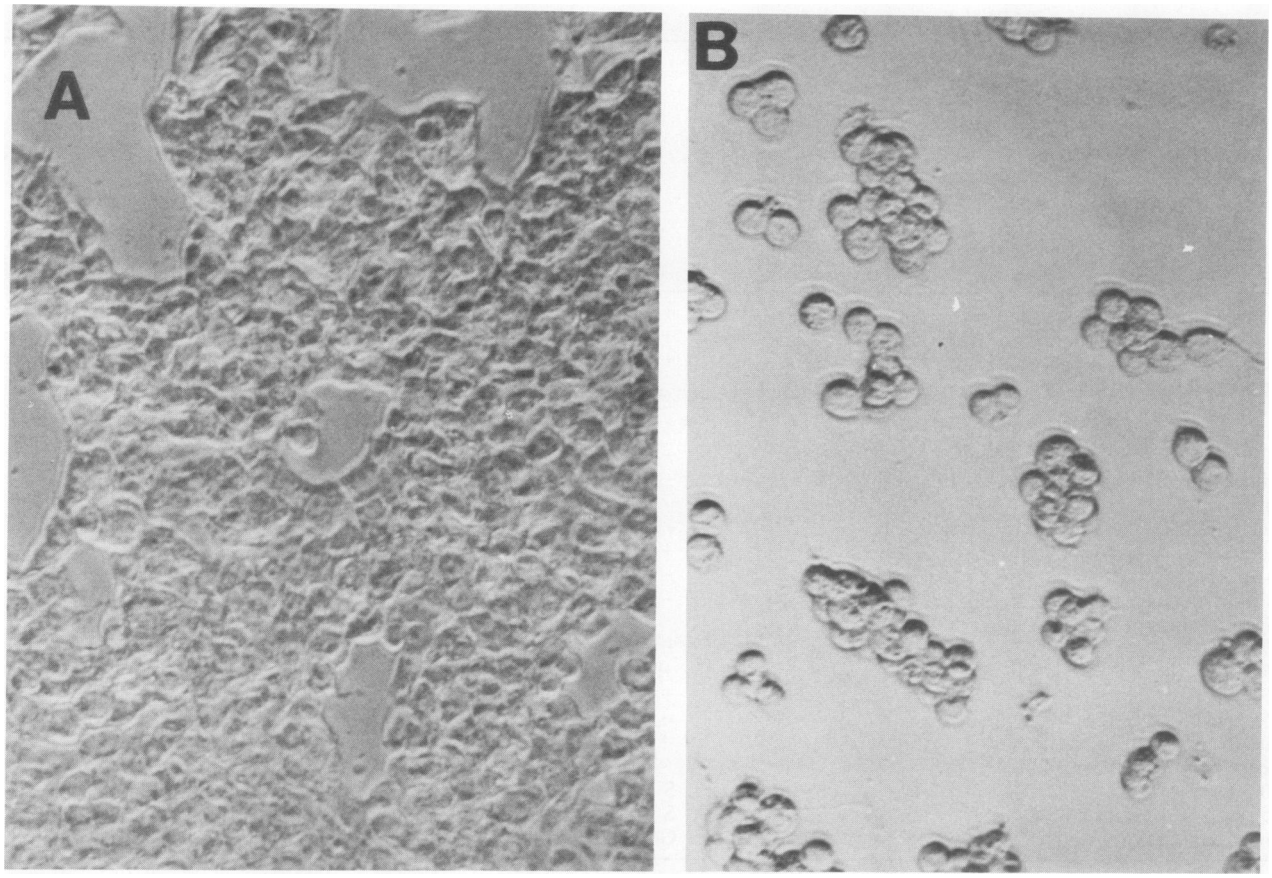

FIG. 1. Y1 adrenal cell assay for cholera toxin. (A) Negative control; (B) cytotonic response elicited by *V. cholerae* 569B.

against the heat-labile enterotoxin with the ELISA. These methods and test procedures (usually performed in reference laboratories) are described in detail by Young et al. (224) in the *Manual of Clinical Laboratory Immunology*.

Musher et al. (139) have demonstrated the presence of bactericidal and opsonizing antibody in the serum of a patient who was treated for, and survived, a serious infection with *V. vulnificus*. Antibodies to the homologous isolate have also been demonstrated by indirect immunofluorescence in a patient with *V. vulnificus* septicemia (156). According to Morris and Black (135), antibody response to non-O1 *V. cholerae* has not been demonstrated, and data are lacking for other species.

Antibiotic Susceptibility

The clinically significant *Vibrio* spp. usually grow well on Mueller-Hinton agar and in the broth used for susceptibility testing, without added NaCl. Addition of NaCl is not recommended since it may alter activity of some antibiotics (65). Tison and Kelly (198) state that susceptibility tests may be performed by disk diffusion, broth or agar dilution, or automated tests.

Most strains of vibrio are susceptible to tetracycline, chloramphenicol, and aminoglycosides (135). Results of 1,009 clinically significant *Vibrio* spp. received by the CDC and tested by the agar diffusion disk method have been reported by Farmer et al. (46). Most strains were susceptible to tetracycline, chloramphenicol, gentamicin, and nalidixic acid, whereas susceptibility to sulfonamides was variable. *V. parahaemolyticus*, *V. alginolyticus*, and *V. furnissii* were resistant to ampicillin, carbenicillin, and cephalothin. Similar findings have been reported by other investigators (91, 135; A. von Graevenitz, Clin. Microbiol. Newslett. **5**:41–43, 1983). Although plasmid-mediated resistance does not seem to be a problem with *V. cholerae* strains seen in the United States, in recent years there have been outbreaks in Africa and Asia with strains resistant to tetracycline, ampicillin, and trimethoprim-sulfamethoxazole (110, 131).

Enterotoxin Testing

A variety of techniques have been used to detect enterotoxins (heat labile and stable) elaborated by vibrios. These methods include animal (rabbit ileal loop, infant rabbit, suckling mouse, mouse lethality, and rabbit permeability factor) and tissue culture (Y1, CHO) assays (Fig. 1), ELISA, latex agglutination, and gene probes. These techniques and others are covered in an excellent review by Wachsmuth (208). As pointed out in her article, none of these procedures lend themselves readily to routine laboratory testing and they are best performed by reference and research laboratories. Briefly, some of the impracticalities in undertaking toxin testing follow. First, optimal cultural conditions for maximal toxin production vary from species to species, and strain variation has been noted. In addition, a number of factors or metabolites affect toxin expression and include the culture medium used, pH, requirement for NaCl, aeration versus static incubation, and addition of lincomycin (101, 105, 121, 144, 160, 202, 220, 221). Animal models are

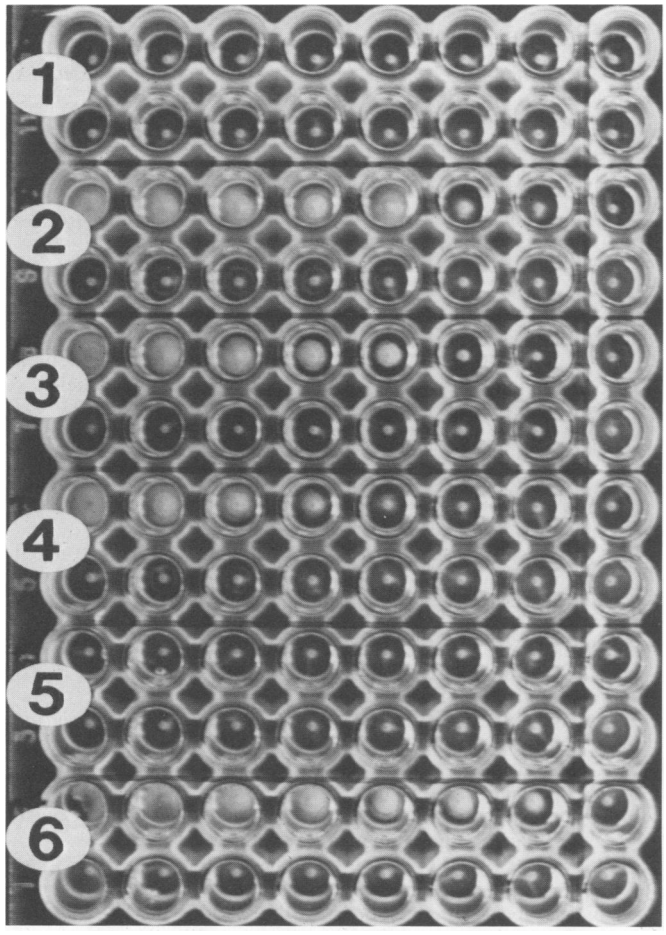

FIG. 2. Filtered supernatants from overnight Evan Casamino Acids-yeast extract broth cultures diluted 1:2 to 1:128 in parallel rows. The last well in each row contains a latex control and diluent only. Latex sensitized with rabbit immunoglobulin G to *V. cholerae* enterotoxin and latex coated with nonimmune rabbit globulin (control) are added to the top and bottom wells, respectively, of each set of parallel rows. Beads covering from one-third (+) to the entire (+++) well surface are positive, while beads in compact buttons are negative for agglutination. Positive samples include *V. cholerae* biotype El Tor, serotype Ogawa (rows 2 and 3), enterotoxin-producing *V. cholerae* non-O1 (row 4), and positive toxin control (row 6). Samples in rows 1 and 5 are negative.

expensive and time-consuming and require animal facilities. Tissue cell assays, sensitive and relatively easy to perform, require cell line maintenance, and the proteases/hemolysins of some vibrio strains may mask the detection of cytotonic enterotoxin activity. Gene probes are specific as well as sensitive, but at this time require the handling of radioactive materials which have a short life span and are not commercially available. The inability to obtain reagents is a drawback of the ELISA methodology as well as of gene probes. ELISA and latex agglutination (Vet-RPLA from Denka Seiken, Tokyo, Japan, and Oxoid Ltd., Hampshire, England) (Fig. 2) are both immunological procedures and may detect inactive, partial toxin (choleragenoid) as opposed to whole toxin.

CONCLUSIONS

The picture that emerges regarding *Vibrio* infections is a fascinating one of an expanding spectrum of clinical syndromes, pathogenic species, and virulence-associated factors attributed to this genus. Despite numerous taxonomic, molecular, and genetic advances made over the past decade concerning this group, many questions still remain. At present it is unclear how often *Vibrio* infections due to the less commonly reported species (other than *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*) occur in humans, particularly in industrialized nations. Further case reports detailing infections with one or more of the less frequently encountered pathogenic members of this group need to be published to broaden our knowledge regarding the range of illnesses and epidemiology associated with each species. In regard to *V. cholerae* non-O1, it remains undetermined whether certain serotypes are inherently more pathogenic than others. For instance, Smith serotype 17 is frequently reported as a cause of gastroenteritis and extraintestinal disease in the United States. Does this clinical incidence indicate a more virulent group or simply reflect their common occurrence in environmental samples? Other than *V. cholerae* O1 and Kanagawa-positive *V. parahaemolyticus*, do described virulence factors correlate with pathogenicity or specific infectious syndromes, or are they found in both pathogenic and nonpathogenic isolates of a given species? One interesting question seldom addressed is the search for similar factors (enterotoxins, toxins, or enzymes) in nonpathogenic environmental vibrios. A study of some of the more commonly occurring members of this group when compared with pathogenic species might shed some light on what elements regulate/contribute to human pathogenicity.

In regard to the environmental surveillance for pathogenic vibrios, particularly from shellfish, virulence markers indicating the inherent pathogenicity of such isolates are generally lacking for all species excluding *V. cholerae* O1 and *V. parahaemolyticus*. Markers for pathogenic *Vibrio* species need to be developed and infective doses need to be determined so that guidelines can be established to evaluate the risk assessment for specified concentrations of each species in raw shellfish meats earmarked for consumption.

In summary, our knowledge regarding the unique association of humans with these aquatic bacteria will continue to increase over the next decade due to progress occurring in a number of these above-mentioned areas. In this way, a better understanding of methods for the prevention and treatment of *Vibrio* infections should be forthcoming.

ACKNOWLEDGMENTS

We thank Sharon Kurashige for her assistance with photographs and Lu-Anne Dodge for typing the manuscript.

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