Sources, Distribution, and Conveyance of Opportunistic Pathogens in Estuaries and the Oceans

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ABSTRACT

In 1981, water samples collected from 14 stations distributed along a track-line from Barbados to Bermuda revealed a preponderance of bacteria belonging to the genus *Vibrio* (Grimes et al. 1984a). Ten years earlier, using the same experimental design along the same approximate track-line, Sieburth (1971) had reported that nearly 100% of the culturable bacterial isolates belonged to the genus *Pseudomonas*. Explanations offered for this apparent shift in the dominance of culturable bacteria included chronic pollution of the ocean with anthropogenic hydrocarbons, resulting in the selection of hydrocarbon-degrading vibrios (Grimes et al. 1984a). It is well known that some species *Vibrio* cause a variety of diseases in marine fishes, marine invertebrates, and humans. *Vibrio* species are also autochthonous inhabitants of estuaries and the oceans, and they possess an array of degradative enzymes that allow them to metabolize compounds ranging from chitin and squalene to short chain fatty acids and polyaromatic hydrocarbons. Clearly, the vibrios possess an impressive set of habitat-adaptive enzymes, and this general adaptation was recently confirmed for one species by the completion of the genomic sequence of *V. cholerae* (Heidelberg et al. 2000). In 1997 and 1998, the largest outbreaks of human disease ever caused by *V. parahaemolyticus* in North America occurred from the consumption of contaminated oysters. Interestingly, these years were also years of increased sea surface temperature associated with the El Niño Southern Oscillation, and *V. parahaemolyticus* grows best under mesophilic conditions. The focus of this paper will be the niches that are filled by members of the bacterial genus *Vibrio* that are capable of degrading anthropogenic compounds, responding to global climate change, and infecting and causing disease in vertebrate and invertebrate hosts.

KEY WORDS: Autochthonous, allochthonous, anthropogenic chemicals, nutrients, pathogens, opportunistic, *Vibrio*, ENSO, currents, degradation

INTRODUCTION

Recent literature abounds with reports of marine diseases affecting marine organisms and humans (Grimes 1991, Harvell et al. 1999, DePaola et al. 2000). After a thorough review of the literature on this matter, Harvell et al. (1999) concluded that reports of diseases in the oceans are on the rise. They noted that there have been many epidemics affecting economically and ecologically important species, and that unknown species may be disappearing without notice. They went

on to note that most "new" diseases occur by host shifts and not by the emergence of "new" microorganisms. Clearly, pathogens have always existed in estuaries and the oceans and they continue to plague humans and the living resources available to humans for sustainable use.

SOURCES AND DISTRIBUTION

Opportunistic pathogens are organisms that do not normally cause disease in a host unless that host has been in some way compromised. There are many such pathogens living in oceans (Grimes et al. 1986) and estuaries (Grimes 1991), some of which normally reside in those habitats and some of which are transients. The indigenous pathogens are referred to as autochthonous by ecologists and their physiology is ideally suited to survival and life in estuaries and the oceans. Unfortunately, there are also mechanisms of attachment and growth that allow these microorganisms to infect susceptible hosts and therein cause disease. Some of the better known examples of autochthonous opportunistic pathogens are listed in Table 1. The non-indigenous pathogens are transients that gain entrance to oceans and estuaries through some point or non-point source of pollution, and they do not usually survive well in these saline aquatic environments. Ecologists refer to non-indigenous microorganisms as allochthonous, and the better known examples of allochthonous opportunists are listed in Table 2.

Table 1. Genera containing opportunistic pathogens autochthonous to estuaries and oceans*

Known autochthonous bacteria	Possibly allochthonous
*Vibrio**	*Staphylococcus*
*Aeromonas**	*Clostridium*
Renibacterium	*Pseudomonas**
*Mycobacterium**	*Flavobacterium*
Plesiomonas	*Klebsiella**
	Acinetobacter
	*Legionella**

*Some members of the genus have demonstrated ability to become nonculturable in response to stress

An important survival mechanism that allows both autochthonous and allochthonous microorganisms to exist in spite of environmental stress was first described by Xu et al. (1982) for *Vibrio cholerae* and *Escherichia coli* - good examples of an autochthonous opportunist and an allochthonous opportunist, respectively. Since that initial description, many laboratories throughout the world have observed the viable but nonculturable phenomenon for numerous pathogens and non-pathogens alike. The essence of this mechanism is illustrated in the survival

curves shown in Figure 1. Briefly, when stressed by a physical or chemical factor (e.g., loss of nutrients, adverse temperature, chlorine), microbes examined thus far respond to the stress by undergoing a series of structural and physiological changes that result in a dormant or "nonculturable" stage of growth. They tend to become smaller, less permeable, refractory to cultivation on culture media normally supportive of their vegetative growth, and some lose their flagella. Little is known about the mechanism or mechanisms that return normal vegetative growth, but it is clear that a susceptible host will suffice for many of the opportunistic pathogens when they are in a nonculturable state. Recently, Colwell and Grimes (2000) collected and published several reviews of this survival strategy that appears to play an important role in the conveyance of opportunistic pathogens in estuaries and oceans. The microorganisms listed in Tables 1 and 2 that have been shown to be capable of this survival strategy are so denoted.

Table 2. Genera containing opportunistic pathogens allochthonous to estuaries and oceans*

Known allochthonous bacteria	Possibly autochthonous
*Salmonella**	*Staphylococcus*
*Shigella**	*Clostridium*
Leptospira	*Pseudomonas**
*Escherichia**	*Flavobacterium*
*Campylobacter**	*Klebsiella**
*Enterococcus**	*Acinetobacter*
Morganella	*Legionella**
*Enterobacter**	
Listeria	

*Some members of the genus have demonstrated ability to become nonculturable in response to stress

Of all the known opportunistic pathogens in estuaries and oceans throughout the world, the *Vibrio* species generally predominate the culturable microbial community (Grimes et al. 1984a). This was an unexpected finding by Grimes et al. (1984a), because since the mid-1900s it was almost axiomatic that *Pseudomonas* species dominated the culturable bacterial communities of the world oceans (ZoBell and Upham 1944, Sieburth 1971). In 1981, water samples collected along a track-line from Barbados east into the Caribbean Sea and north through the Mona Passage to Bermuda demonstrated that vibrios had replaced pseudomonads (Grimes et al. 1984a). *Vibrio* species comprised up to 100% of the culturable community, followed by acinetobacters (Figure 2, Grimes et al. 1984a). Now, it is known that *Vibrio* species are ubiquitous in oceans and estuaries – throughout the water column, in bottom sediments, attached on marine plants and animals, and within marine animals. Their distribution and density are, ultimately, dependant upon known environmental limiting factors, including salinity (vibrios preferentially generate

energy by means of a Na-dependant ATP pump), temperature (vibrios are mesophiles), organic and inorganic nutrients (all vibrios are chemoheterotrophs, some can fix N, and most can use nitrate in anaerobic respiration), currents (bacteria are colloids that are easily conveyed by tides, currents, upwellings, and rings), and possibly light.

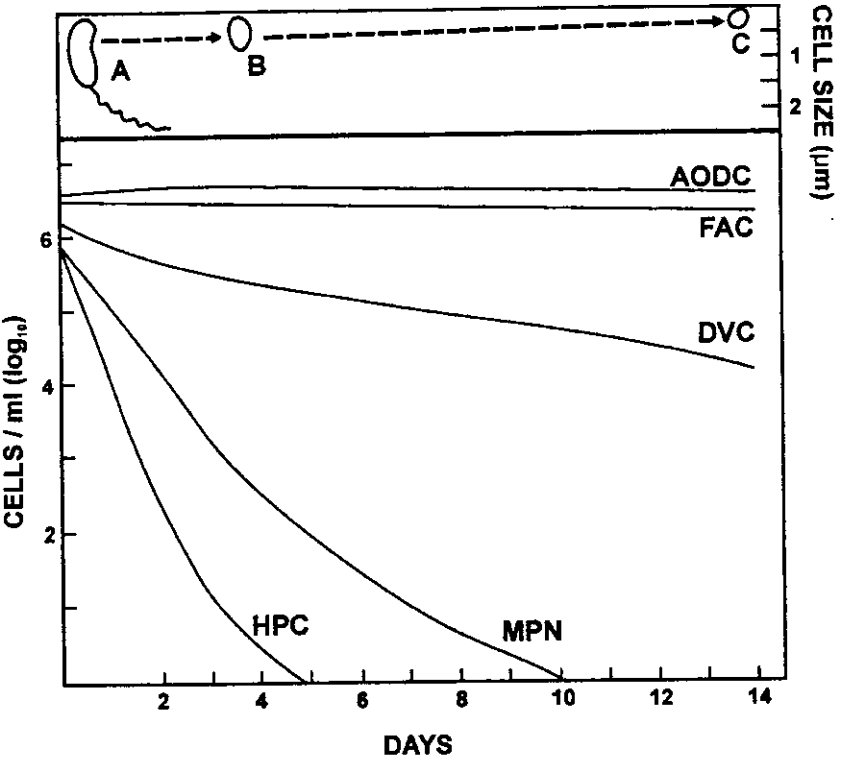

Figure 1. Survival curves and morphological changes typical of vibrios as they transition from vegetative growth into the viable but nonculturable stage of growth

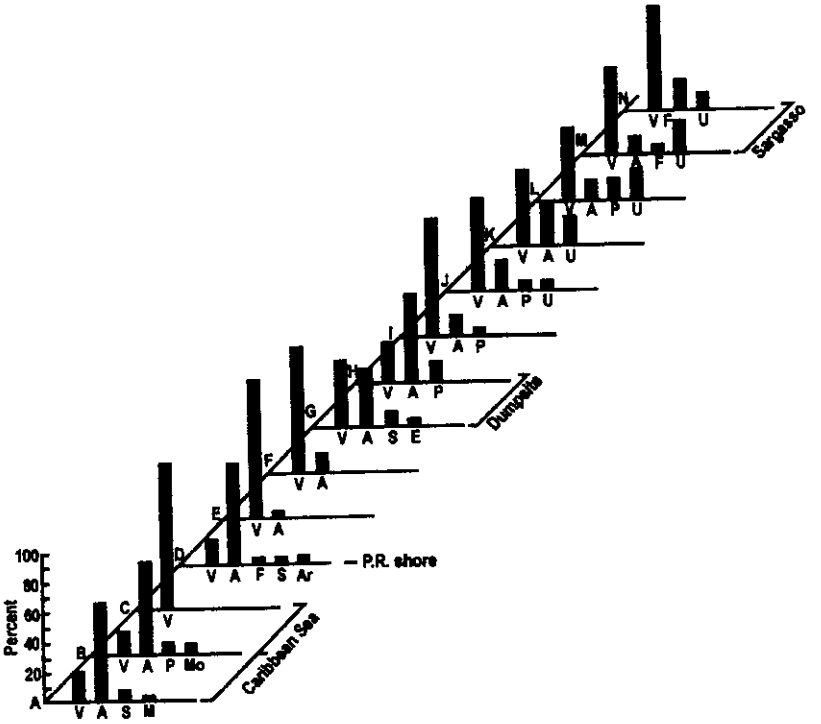

Figure 2. Distribution of culturable, heterotrophic bacteria in water samples collected at 14 stations along a track-line from Barbados, into the Caribbean Sea, and through Mona Passage into the Atlantic Ocean to Bermuda

Consistent with their ubiquitous distribution in oceans and estuaries, vibrios and acinetobacters are capable of metabolizing a wide variety of hydrocarbons. Short chain volatile fatty acids, alcohols, toluene, benzene, dimethylaniline, and polyaromatic hydrocarbons (PAHs) are all subject to biodegradation by vibrios and acinetobacters (Okpokwasili et al. 1986), and these hydrocarbons were characteristic of wastes being disposed of at the Puerto Rico dumpsite and of petroleum being transported from South America (Grimes et al. 1984a). Clearly, the vibrios are capable of metabolizing a wide variety of anthropogenic chemicals.

Vibrios are also capable of metabolizing a wide variety of biopolymers. Most can hydrolyze and utilize monomers from proteins, complex carbohydrates, and lipids. This ability not only confers metabolic versatility on the vibrios, but it also allows them to colonize and grow in a variety of animal hosts. They can hydrolyze the building blocks of connective and soft tissues; in other words, they can easily exploit the habitat provided by chance hosts and act as opportunistic pathogens. Table 3 illustrates the metabolic versatility of the vibrios, by listing some of the compounds that can be used by the shark pathogen *V. carchariae* (Grimes et al. 1984c, Grimes et al. 1989).

Table 3. Compounds metabolized by *Vibrio charchariae*, an opportunistic pathogen of sharks (Grimes et al. 1984c and 1989)

Lysine, ornithine, tryptophan, glycine	Starch, algin
Chitin, chondroitin, hyaluronic acid	Xanthine, salicin
Casein, gelatin, collagen	γ-aminobutyrate
Lecithin, squalene	α-ketoglutarate
Tweens 20, 40, 60, 80	Glucose, sucrose, mannose
Urea	Trehalose, arabinose, mannitol
Nitrate	Ethanol, 1-propanol

Clearly, the vibrios are elegantly equipped to quickly exploit a wide variety of potential nutrient sources - inputs and fluxes - in estuaries and oceans. The full extent of this capability was recently demonstrated for the type species of the genus, *V. chloerae*, by Heidelberg et al. (2000). The vibrios closely follow primary producer as well as primary consumer population increases, as they grow in response to fluxes in dissolved organic material and on living and dead zooplankton (Harvell et al. 1999, Kaneko and Colwell 1973). They respond to anthropogenic inputs (Grimes et al. 1984a) and to climate change (Lobitz et al. 2000). In every sense, they are "r-strategists" or copiotrophs, living off the "fat of the land" (Grimes 1991). Vibrios grow quickly in response to new sources of nutrients in natural aquatic habitats, they rapidly disseminate in compromised hosts, and they become dormant or nonculturable (Colwell and Grimes 2000) during famine or otherwise adverse environmental conditions.

CONVEYANCE

There is ample evidence that vibrios, like most marine bacteria, are conveyed throughout estuaries and oceans of the world by both natural and anthropogenic influences. Currents, tides, waves, gyres, and other natural water movements convey bacteria as they would any comparable sized particle. In 1984, it was hypothesized that pharmaceutical wastes released at a dumpsite north of Puerto Rico meandered throughout the Atlantic Ocean and, eventually, into the Caribbean Sea (Grimes et al. 1984a). In turn, it was further hypothesized that these wastes stimulated the growth of a community of vibrio bacteria in the affected waters. In a related study, it was shown that pharmaceutical wastes introduced into the ocean by means of a long sewage outfall also had an influence that was related to prevailing currents (Grimes et al. 1984b).

In 1997, consumption of raw oysters from the Pacific Northwest resulted in a major disease outbreak of *V. parahaemolyticus* that caused 210 cases, including one death from septicemia (CDCP 1998). The *V. parahaemolyticus* strains involved (O1, O4, and O5) were serogroups commonly found in the U.S., and there was reason to believe that their increased prevalence in oysters harvested from Pacific NW waters may have been associated with increased water temperatures caused by the El Niño Southern Oscillation. The following year, *V. parahaemolyticus* appeared again, this time predominantly in oysters harvested from Galveston Bay, Texas, and it exclusively involved an Asian serogroup (O3:K6) not reported from the U.S. since 1972 (DePaola et al. 2000). The 1998 "*Vp*"outbreak was the largest ever recorded in the U.S., involving over 500 individuals but no deaths. This time, there was reason to believe that the exotic "*Vp*" may have been introduced through ship ballast water.

CONCLUSIONS

It has been documented that a shift in the culturable community of heterotrophic bacteria in the Atlantic Ocean occurred sometime in the late 1970s. A variety of influences may have caused this shift, including anthropogenic nutrients – both organic and inorganic. The resulting "climax" bacterial community is characterized by a predominance of *Vibrio* species, including autochthonous vibrios pathogenic for humans and fishes. Global climate change may have also influenced, either directly or indirectly, this bacterial community-shift. It is hypothesized that the chemicals which cause bacterial community-shift also stress potential animal hosts (e.g., fishes), thereby allowing the increased densities of opportunistic pathogens to easily invade, colonize, and cause disease in their hosts. With regard to human hosts, demographics (e.g., most people live close to the coastal ocean) and food preferences (e.g., seafood is becoming increasingly popular) are also contributing to risk. Overall, it would appear that anthropogenic influences – inorganic nutrients, toxic chemicals, organic chemicals, greenhouse gases, global transportation – are influencing the sources, distribution, and conveyance of opportunistic pathogens in estuaries and the oceans.

LITERATURE CITED

Centers for Disease Control and Prevention (CDCP). 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters – Pacific Northwest, 1997. *Morbidity and Mortality Weekly Report* **47**:457-462.

Colwell, R.R. and D.J. Grimes (eds.). 2000. *Nonculturable Microorganisms in the Environment*. ASM Press. Washington, D.C. USA. 354 pp.

DePaola, A., C.A. Kaysner, J. Bowers, and D.W. Cook. 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Applied Environmental Microbiology* **66**:4649-4654.

Grimes, D.J. 1991. Ecology of estuarine bacteria capable of causing human disease: a review. *Estuaries* **14**:345-360.

Grimes, D.J., F.L. Singleton, and R.R. Colwell. 1984a. Allogenic succession of marine bacterial communities in response to pharmaceutical waste. *Journal of Applied Bacteriology* **57**:247-261.

Grimes, D.J., F.L. Singleton, J. Stemmler, L.M. Palmer, P. Brayton, and R.R. Colwell. 1984b. Microbiological effects of wastewater effluent discharge into coastal waters of Puerto Rico. *Water Research* **18**:613-619.

Grimes, D.J., J. Stemmler, H. Hada, E.B. May, D. Maneval, F.M. Hetrick, R.T. Jones, M. Stoskopf, and R.R. Colwell. 1984c. *Vibrio* species associated with mortality of sharks held in captivity. *Microbial Ecology* **10**:271-282.

Grimes, D.J., R.W. Attwell, P.R. Brayton, L.M. Palmer, D.M. Rollins, D.B. Roszak, F.L. Singleton, M.L. Tamplin, and R.R. Colwell. 1986. Fate of enteric pathogenic bacteria in estuarine and marine environments. *Microbiological Sciences* **3**:324-329.

Grimes, D.J., J. Burgess, J.A. Crunkleton, Jr., P.R. Brayton, and R.R. Colwell. 1989. Potential invasive factors associated with *Vibrio carchariae*, an opportunistic pathogen for sharks. *Journal of Fish Diseases* **12**:69-72.

Harvell, C.D., K. Kim, J.M. Burkholder, R.R.Colwell, P.R. Epstein, D.J. Grimes, E.E. Hoffmann, E.K. Lipp, A.D.M.E. Osterhaus, R.M. Overstreet, J.W. Porter, G.W. Smith, and G.R. Vasta. 1999. Emerging marine diseases - climate links and anthropogenic factors. *Science* **285**:1505-1510.

Heidelberg, J.F. et al. 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* **406**:477-483.

Kaneko, T. and R.R. Colwell. 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Journal of Bacteriology* **113**:24-32.

Lobitz, B., L. Beck, A. Huq, B. Wood, G. Fuchs, A.S.G. Faruque, and R.R. Colwell. 2000. Climate and infectious disease: Use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. *PNAS* **97**:1438-1443.

Okpokwasili, G.C., C.C. Somerville, M. Sullivan, D.J. Grimes, and R.R. Colwell. 1986. Plasmid mediated degradation of hydrocarbons in estuarine bacteria. *Oil and Chemical Pollution* **3**:117-129.

Sieburth, J.McN. 1971. Distribution and activity of oceanic bacteria. *Deep Sea Research* **18**:1111-1121.

Xu, H.-S., N. Roberts, F.L. Singleton, R.W. Attwell, D.J. Grimes, and R.R. Colwell. 1982. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microbial Ecology* 8:313-323.

ZoBell, C.E. and H.C. Upham. 1944. A list of marine bacteria including descriptions of sixty new species. *Bulletin of the Scripps Institute of Oceanog.raphy. University of California Technical Series* 5:239-292.