Environmental Predictors of Pathogenic Vibrios in South Florida Coastal Waters

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Abstract: In 2007, diseases caused by *Vibrio vulnificus* and other *Vibrio* species became nationally notifiable in the United States because of the potential severity of bloodstream infections. Direct contact of open wound with seawater and the ingestion of contaminated oysters are the principal modes of transmission. Presently, no clear environmental predictors of oyster contamination are known. This study is the first to report an apparent association between rainfall and *Vibrio* counts at five South Florida beaches. Using multiple regression and ANOVA, the relationship between *Vibrio* populations and various environmental factors were examined. *Vibrio* counts ranged from 135 CFU/100 mL at Hollywood Beach to 186,000 CFU/100 mL at North Miami Beach. *Vibrio* v*ulnificus* and *parahemolyticus* were detected (less than 1% of all identified isolates) at two and four beaches respectively. Temperature and rainfall dates were the most significant correlates of the incidence of pathogenic *Vibrio* species.

Keywords: Vibrio prevalence, environmental factors, rainfall, pathogenic vibrios, coastal beaches.

INTRODUCTION

Prior to 2007, the United States lacked a national surveillance system for *Vibrio vulnificus* infections. Although only about 900 cases were reported between 1988 and 2006 in the Gulf coastal states [1], the potential severity and high mortality of bloodstream infections have launched these pathogens into the limelight. The dearth of information on the ecological dynamics of *V. vulnificus* in response to environmental variables makes it difficult to predict transmission risks *via* oyster consumption.

Vibrio species are ubiquitous to marine, estuarine, and freshwater environments [2]. Utilizing existing wounds [3, 4] and ingestion of contaminated water or seafood [5] as their major routes of infection, vibrios can cause enteric diseases, wound infections, and bloodstream invasion in vulnerable individuals [6, 7]. According to Howard and Bennett [6], prevalence of marine *Vibrio* infections (foodborne and waterborne) in Florida ranks among the highest in the world, and swimmers in coastal waters of the Gulf of Mexico are also affected. Water quality standards established by the U.S. Environmental Protection Agency (USEPA) [8] for monitoring recreational waters (using *Escherichia coli* and enterococci as indicators) do not always correlate with the incidence or infectious doses of pathogenic vibrios [9-13]. In addition, Bonilla *et al.,* [14] noted that total coliform and fecal coliform numbers were not perfect predictors of diarrheal diseases of swimmers in marine waters. Knowledge of the prevalence of pathogenic *Vibrio* species and the environmental factors that affect their numbers in recreational beaches is important for risk assessment and control of disease transmission *via* oyster beds.

Here, we report the detection and relative abundance of pathogenic *Vibrio* species in five South Florida beaches. The influence of salinity, rainfall, and water temperature on the occurrence of these bacteria was also evaluated.

MATERIALS AND METHODS

Seawater from five popular beaches on the southeast coast of Florida (Pompano Beach at Atlantic Blvd., Fort Lauderdale Beach at Las Olas Blvd., Hollywood Beach at Johnson St., North Miami Beach between 182nd and 183rd Streets., and South Beach at 9th St.) was sampled every two weeks, from October 9th to November 19th 2007, between 7:30 am and 11:30 am. At each sampling site, the water was obtained in duplicate from about 30 cm below the water surface in 100-mL sterile sample bottles. Water temperature and salinity were measured at each site. The rainfall data at each location was obtained from District Daily Rainfall Report of South Florida Water Management District (www.sfwmd.gov/curre/3_rainfall.html).

Total cultivable *Vibrio* (TCV) counts were determined by the membrane filtration technique [15] using 45μm-pore size membranes (Millipore, Waltham, MA) on Difco™ Thiosulfate-Citrate-Bile-Sucrose (TCBS) Agar plates (Becton Dickinson, Franklin Lakes, NJ). Plates were incubated at room temperature (22-24°C) for 24 hours. Characteristic *Vibrio* colonies were counted and expressed as colony forming units (CFU) 100 mL^{-1} beach water sample.

Characteristic *Vibrio* colonies on TCBS Agar were isolated and purified on modified Tryptic Soy Agar (TSA) (Becton Dickinson, Cockeysville, MD) - 5% pancreatic digest of casein, 0.5% soy bean meal, 3% NaCl, 0.15% $MgSO_4$, and 1.5% Agar. The isolates were definitively identified with Biolog GN Microplate system (Biolog Inc.,

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1874-2971/12

Hayward, CA) according to manufacturer's instructions except that overnight cultures of the strains were grown on the amended TSA. Statistical analyses, including one-way ANOVA, regression analysis, and Pearson correlation (Minitab 13, Minitab Inc., State College, PA) were used to test the relationship between the various factors measured.

RESULTS AND DISCUSSION

Table **1** displays the dynamics of *Vibrio* bacteria in the recreational beaches studied. *Vibrio alginolyticus*, *V. carchariae* (*harveyi*) and *V.* species were the most prevalent in all beaches studied. Notably, *V. vulnificus* and *V. parahaemolyticus*, which are typically the most frequently encountered pathogenic vibrios, were rarely present on the sample dates and detected at two and four beaches respectively. They were mainly found together at Pompano and North Miami beaches, at very low levels (1% of the total *Vibrio* population). On three of four sampling dates, *V. vulnificus* occurred at North Miami Beach- a phenomenon that may be linked to nutrient status (unpublished data), beach topography, and run-off.

Such low densities of *V. vulnificus* and *V. parahaemolyticus* at the studied beaches suggest these pathogenic vibrios may reside in other niches along Florida's vast coastline. It is possible that the majority of *Vibrio* sources in Florida are derived from the West coast, along the Gulf of Mexico. In a US study surveying non-foodborne marine *Vibrio* infections from 1997-2006, states along the Gulf Coast reported the largest number of cases (57%), followed by those in the Atlantic region (24%) [16].

Despite the low occurrence of pathogenic vibrios at the studied Florida beaches, their mere presence can easily translate into a risk of foodborne infection as they tend to accumulate in filter-feeding organisms such as oysters and clams [6]. It is also worth noting that the risk of non-foodborne infections by marine vibrios may greatly be influenced by individual immune status and exposure to open wounds.

A negative correlation (Pearson correlation = -0.540, P=0.014) was found between total *Vibrio* counts and total coliforms (results not shown). Temporal variation at a given

Table 1. Occurrence of *Vibrio* Species in Five South Florida Beaches

Sampling Date	Beach Location	Water Temperature (°C)	†Rainfall (Inch)	Salinity (ppt)	Total Cultivable Vibrio (Log CFU/100 mL)	*V. alginolyticus*	*V. carchariae (harveyi)*	≠*V. parahaemolyticus*	*V. vulnificus*
Oct. 11	Pompano (Atlantic Blvd.)	28.0	0.35	32.2	3.38	++	+	*	nd
	Ft. Lauderdale (Las Olas Blvd.)	28.0	0.42	32.0	3.01	++	+	nd	nd
	Hollywood (Johnson St.)	28.0	1.06	32.5	2.13	++	+	nd	nd
	North Miami (182nd and 183rd St.)	27.5	0.48	32.5	2.43	++	+	nd	*
	South Beach (9th St.)	27.0	0.22	33.0	2.23	++	+	nd	nd
Oct. 23	Pompano (Atlantic Blvd.)	26.0	0	32.0	3.88	++	+	*	*
	Ft. Lauderdale (Las Olas Blvd.)	26.5	0	32.8	5.09	++	+	*	nd
	Hollywood (Johnson St.)	26.8	0.02	32.9	4.12	++	+	nd	nd
	North Miami (182nd and 183rd St.)	26.5	0.46	31.1	4.28	++	+	nd	nd
	South Beach (9th St.)	26.0	0	31.1	4.32	++	+	nd	nd
Nov. 6	Pompano (Atlantic Blvd.)	25.0	0.10	31.0	4.06	++	+	*	nd
	Ft. Lauderdale (Las Olas Blvd.)	24.5	0	31.0	4.46	++	+	*	nd
	Hollywood (Johnson St.)	25.0	0	31.0	4.32	++	+	nd	nd
	North Miami (182nd and 183rd St.)	24.0	0	32.5	5.27	++	+	*	*
	South Beach (9th St.)	25.0	0	32.5	4.49	++	+	nd	nd
Nov. 20	Pompano (Atlantic Blvd.)	24.5	0	33.0	4.42	++	+	*	nd
	Ft. Lauderdale (Las Olas Blvd.)	24.0	0	32.5	4.57	++	+	*	nd
	Hollywood (Johnson St.)	24.0	0	32.5	4.02	++	+	nd	nd
	North Miami (182nd and 183rd St.)	23.0	0	34.5	4.54	++	+	*	*
	South Beach (9th St.)	23.5	0	34.0	4.18	++	+	*	nd

† Source of rainfall data: www.sfwmd.gov/curre/3_rainfall.html; ++ = More than 70% of recovered vibrios; + = Less than 20% of recovered vibrios; * = Less than 1% of recovered vibrios; ≠ = *V. parahaemolyticus* and other *Vibrio* species; nd = Not detected.

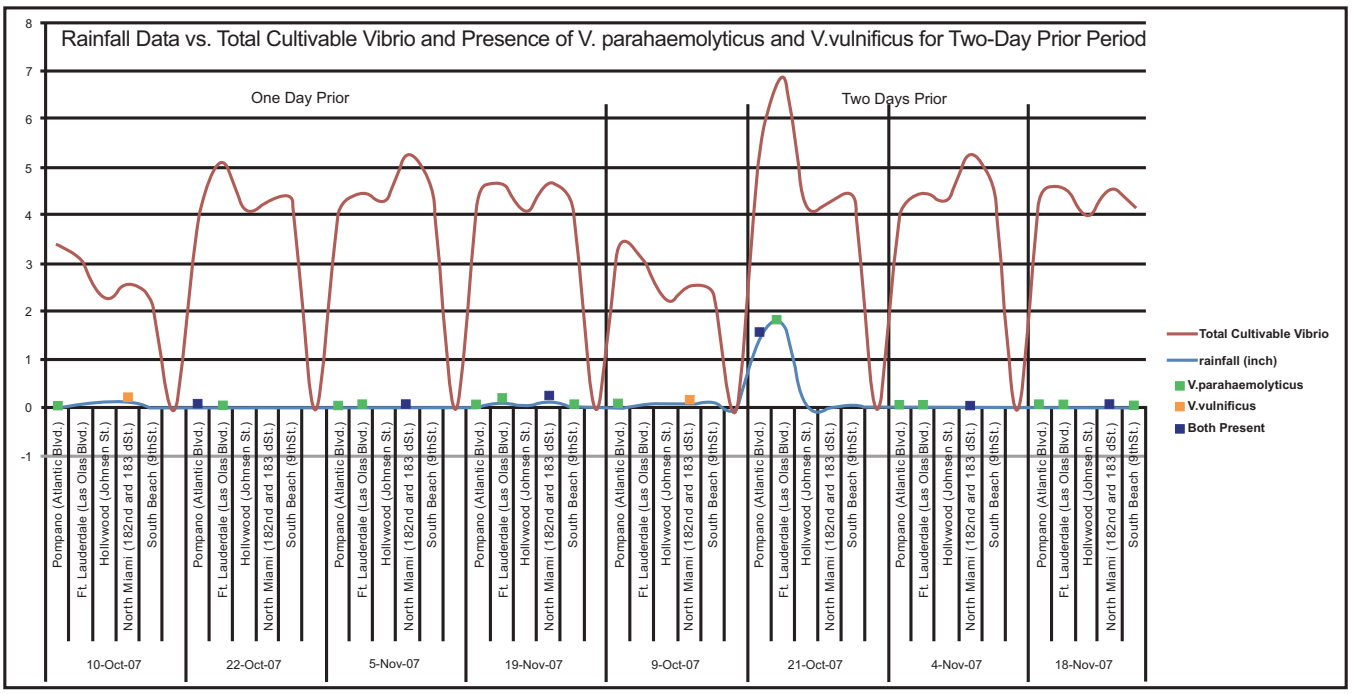

Fig. (1). Occurrence of total cultivable and pathogenic vibrios in five public beaches of South Florida plotted against rainfall data from one and two days prior to sampling.

site was highly significant (F=33.26, P=0.001). Several possible factors that might influence the densities of *Vibrio* bacteria were selected and examined by regression analysis.

Three rainfall time-points including: two days prior to sampling (day -2); one day before (day -1); and the day of sampling (day 0), were analyzed against CFU/100mL of seawater at each location and sampling date. Remarkably, the level of vibrios in the coastal waters demonstrated a significant positive correlation with the occurrence of rainfall two days prior (day -2) to sampling (R^2=26.8%, F=6.60, P=0.019) (Fig. **1**). All other combinations of rainfall and bacterial density showed no relationship. Consequential decrease in salinity, increase in turbidity, change in chlorophyll *a* levels [17], and nutrient accumulation during rainfall runoff may serve as plausible explanations of the observed trend.

Water temperature remained optimal [18] for the survival of vibrios during the study (23-28°C) (Table **1**). In a Florida study surveying the cases of invasive infections due to marine *Vibrio* bacteria between January 1979 and December 1991, 80% of patients developed invasive marine *Vibrio* infections during the summer months of May-October [6], which coincide with increased temperatures and rainfall. The prime climatic conditions for marine *Vibrio* survival at Florida's beautiful beaches could contribute to the elevated incidence of *Vibrio* infections relative to other coastal regions in the United States. Other factors, such as depths of sampling sites and tidal levels are also known to significantly affect the concentration of *Vibrio* spp [13].

CONCLUSION

Total cultivable *Vibrio* counts in the beaches fluctuated remarkably with environmental conditions such as rainfall, especially occurring two days prior to sampling. Levels of the pathogenic vibrios, *V. vulnificus* and *V. parahaemolyticus*, could not be predicted by total *Vibrio* counts. Their incidence also remained very low at the beaches where they were detected. Routine monitoring of South Florida beaches for *Vibrio* numbers does not appear to be warranted given the sporadic occurrence of the pathogens. Occasional assessment of risk after rainfall may, however, be necessary, particularly in areas where oysters are harvested for consumption.

ACKNOWLEDGEMENTS

Mrs. Corine M. Stinson is acknowledged for her contributions in revising and editing this manuscript.

CONFLICT OF INTEREST

There are no known conflicts of interest in this study.

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Received: January 20, 2012 Revised: February 7, 2012 Accepted: February 15, 2012