OCCURRENCE OF NON-O1/NON-O139 VIBRIO CHOLERAE AND AEROMONAS SPP. IN ARIZONA RECREATIONAL WATERS

By

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Abstract

Vibrio cholerae and *Aeromonas spp.* are thermotolerant bacteria that can cause nonepidemic diarrheal diseases and opportunistic skin infections. *Aeromonas* is currently on the Environmental Protection Agency's Contaminant Candidate List (CCL) and although *V. cholerae* is commonly found in marine and estuarine waters, its occurrence in freshwaters is less well understood. In the summer of 2006, two individuals contracted illnesses caused by *V. cholerae* (non-O1/non-O139) while swimming near the Gila River in Eastern Arizona. In the current study, a survey of recreational waters in Arizona was conducted to determine the occurrence of non-O1/non-O139 *V. cholerae* and *Aeromonas spp*. Water samples from various recreational sources were concentrated by membrane filtration and plated on TCBS agar and *m*-Aeromonas selective agar base (Havelaar) for the recovery of *V. cholerae* and *Aeromonas*, respectively. Characteristic bacteria colonies were confirmed by either API 20E (*V. cholerae*) or API 20NE (*Aeromonas*). In addition, total coliforms, *Escherichia coli* and heterotrophic plate count bacteria were quantified; water parameters such as temperature, pH, turbidity and conductivity were also recorded. Thus far, approximately 51% of recreational waters have tested positive for *V. cholerae* and 71% have tested positive for *Aeromonas spp*. These opportunistic pathogens may therefore represent an unrecognized health threat to recreational bathers in Arizona.

Introduction

Non-01/non-0139 *Vibrio cholera* are distinct serotypes of *Vibrio cholera* that do not cause epidemic cholera as does 01 and 0139 serotypes. However, they are important water-borne pathogens that can cause gastrointestinal disease and skin infections. In the summer of 2006, the Arizona Department of Environmental Quality (ADEQ) began studying the occurrence of non-O1/non-O139 *Vibrio cholerae* in recreational waters when two individuals contracted illnesses caused by it while swimming in the Gila River. In August and September of 2006, 23 of 24 water samples taken from 14 sites indicated the presence of non-O1/non-O139 *V. cholera*. This was a unique situation because *Vibrio cholera*e associated illnesses are commonly found in marine/estuarine waters and only rarely freshwaters. We postulate that warm and alkaline recreational waters of Arizona provide an unusual habitat for non-01/non-0139 *Vibrio cholera.*

Beginning in the summer of 2007, the survey of non-O1/non-O139 *V. cholera* in Arizona recreational waters was extended by Dr. Charles Gerba of the University of Arizona and included *Aeromonas spp.* as an organism of interest. *Aeromonas spp* is also a water-borne pathogen that can cause similar gastrointestinal diseases. At the conclusion of the research, we plan to develop a risk assessment of disease from *V. cholerae* and *Aeromonas* when individuals come in contact with recreational waters in Arizona waters. The study is planned to conclude early 2010.

Vibrio cholerae (non-O1/non-O139) is a gram negative curved rod which typically inhabits waters with a pH of 7.8 to 9.1. Non-O1/non-O139 serotypes are distinct from serotypes O1 and O139, which cause epidemic cholera. Due to its predilection for waters of higher pH and warmer temperatures, *Vibrio cholerae* is a threat in marine or estuarine environments, but generally not in surface waters. Non-O1/non-O139 *V. cholerae* cause isolated or small outbreaks

of gastrointestinal disease, which are cholera-like but cause less severe watery diarrhea. The bacteria can also infect blood, wounds, ears, and the respiratory tract (Shannon and Kimbrough 2006). The contraction of the bacteria is often associated with seafood consumption, exposure to polluted fresh water, brackish water, seawater, and foreign travel (Nair et al. 1991; Twedt et al. 1981).

Aeromonas spp. is a gram-negative, facultative anaerobic rod that is almost ubiquitous in aquatic environments worldwide. They have been found in lakes, rivers, reservoirs, groundwater, wastewater, and treated drinking water (Embrey 2002). The bacteria thrive at warmer temperatures of 37°C but are resistant to refrigeration temperatures. In humans the most prominent species that cause infection are *hydrophila*, *sobria*, and *caviae*. Similar to *V. cholerae*, infected individuals experience gastroenteric, cholera-like rice water stool, dysenteric gastroenteritis, blood/mucus stool, and/or cellulitis (Burke et al. 1984; Gavriel et al. 1998). Illness is often associated with exposure to high-nutrient waters (e.g., sewage contamination).

In a broader aspect water-borne pathogens are of interest because global climate change is expected to increase rainfall and water runoff from storms. Changes in precipitation may lead to greater contamination of surface waters and greater waterborne disease outbreaks (Rose et al. 2000). Non-01/non-0139 *Vibrio cholera* and *Aeromonas spp.* may be a factor in future waterborne disease outbreaks.

Materials & Methods

Water Quality Assessment:

Freshwater samples were collected in 1L sterile bottles from various recreational waters in Arizona including lakes, rivers, and reservoirs. Samples were taken preferably near areas with likely human access (e.g., swimming areas). After the samples were taken, the 1L bottles were transported on ice back to the laboratory. The following water quality parameters were tested: temperature, pH, turbidity, chlorine residual, specific conductance, coliform bacteria, heterotrophic bacterial plate count bacteria (HPC), and *E. coli* using the methods listed in Table 4. The number of total coliforms and *E. coli* was determined using the Most Probable Number (MPN) method using sterile 100 ml bottles containing Colilert substrate. The samples will be placed in Quantitrays and sealed using a Quantitray Sealer (IDEXX Laboratories, Westbrook, ME) and then incubated for 24 hours at 37ºC. They will then be examined for the presence of yellow coloration (total coliforms) and fluorescence under a UV light (*E. coli*). Heterotrophic bacterial assays will be performed in triplicate via the spread plating method on R2A media (Difco, Sparks, MD) with incubation at 28^{o}C (room temperature) for 5 days.

Bacterial testing

For *V. cholerae* and *Aeromonas spp.*, an entire one-liter sample will be concentrated in 1ml, 10ml, and 100ml, and 500mL aliquiots using .45 µm membrane filtration. The membranes will then be directly place on thiosulfate citrate bile salts sucrose (TCBS) selective medium for *V. cholerae* and *m*-Aeromonas agar for *Aeromonas spp.* (Lesmana *et al.* 1985; McLaughlin 1995). Any resultant distinctive yellow colonies with a diameter of 1 to 3 mm will then be

subcultured via streaking for isolation on TCBS agar plates for purification. Oxidase test will be performed and positive tests samples were then identified using the API 20E biochemical system for for *V. cholerae* and API 20NE biochemical system for *Aeromonas spp.* (Biomerieux, France) according to the manufacturer's instructions. *V. cholerae* isolates will then be sent to a commercial laboratory for serotyping.

Results & Discussion

Bacterial data of *Vibrio cholerae* and *Aeromonas spp*. in Arizona recreational waters were collected to identify general occurrence since both organisms are suspected of causing human health hazards. The bacteria and water parameter sampling was separated out to cold (Nov. – Feb.) and warm months (May- Oct.) as shown in Table 2 because it demonstrates the seasonal differences in Arizona and separates out peak months of recreational activity generally occurring in warm months. . Thus far, approximately 51% of recreational waters have tested positive for *V. cholerae* and 71% have tested positive for *Aeromonas spp*. The bacterial parameters of warm and cold seasons were subjected to ANOVA analysis according to Table 3. The ANOVA analysis showed significant difference in only HPC alone of p=0.02. Other notable differences in the means were *Vibrio cholera* p=0.28 and Coliforms p=0.11. The differences between these two parameters may be due to lack of sample size.

The prospective goal of the study is to develop a risk assessment of disease from *V. cholerae* and *Aeromonas* when individuals come in contact with recreational waters. If *V. cholerae* and *Aeromonas* are found to be significant risk factors, simpler measures will be used to predict disease incidence. To test the significance of the water parameters, ANOVA, correlations and other statistical tests will be used to determine if water quality parameters such as the presence of fecal indicators (coliforms, E. coli), HPC numbers, temperature, pH, turbidity, rainfall, etc. can be used to predict pathogen numbers and thus the risk of contracting disease. More sample numbers are expected to strengthen apparent relationships found in this study.

In a broader picture, the data may be extrapolated to include other recreational waters from across the United States. Global climate change in the future may increase the risk of

infection from thermotolerant species such as *V. cholerae* and *Aeromonas* due to increases in water temperature and precipitation.

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Table 1. Sample Analyte List and Test Method. Each water quality parameter was measured using the following test methods.

Water Quality Parameter	Test Method
Temperature ºC	Hydrolab Quanta Tester. Standard Methods 2550B p. 2-61 (APHA 2005).
pH	Hydrolab Quanta Tester. Standard Methods 2310Bp. 2-24/ or equivalent (APHA 2005).
Turbidity	HF Scientific Inc./DRT-15CE Portable Turbidity Meter. Standard Methods 2130B p. 2-9 (APHA 2005).
Chlorine Residual	Hach Test Kit #2231-01. Standard Methods 4500D p.4-76 (APHA 2005).
Specific Conductance	Direct measure. Standard Methods 2520B p. 2-45 (APHA 2005).
Coliform Bacteria	Colilert Test, Colorimetric. Standard Methods 9213D p.9-69 (APHA 2005).
Heterotrophic Bacteria	Heterotrophic Plate Count. Standard Methods 9215A/ 9215C p. 9-34 (APHA 2005).
Escherichia coli	Colilert Test, Colorimetric. Standard Methods 9222 p.9-69 (APHA 2005).

Table 2. Compiled Water and Bacterial Parameters of Warm and Cold Seasons. Bacterial and Water parameter data was compiled and separated out to warm (May-Octorber) and cold (November-February) months of Arizona.

Sampling Period	Vibrio cholerae (cfu/100mL)	Aeromonas (cfu/100mL)	Coliforms (MPN/100mL)	E. coli (MPN/100mL)	HPCs (cfu/mL)	Temp. (dC)	pH	Turbidity (NTU)	Conductivity (Uohm/cm)
May-October									
Average	1940	41750	1706	12	5195180	27.6	7.87	12.89	925.1
Geometric Mean	1216	38180	1106	3	79220	27.1	7.85	3.8	647.2
November-February									
Average	23	56127	1030	10	10916	15.2	7.95	7	1155.5
Geometric Mean	16	23882	679	5	1120	15	7.94	3.7	1107.7

Table 3. ANOVA Analysis of Bacterial Parameters in Warm and Cold Seasons. The bacterial parameter differences in warm (May-Octorber) and cold (November-February) months of Arizona was subjected to ANOVA analysis.

Bacterial Parameter	May - Oct	Nov. - Feb.	*P-value*	P(T<=t) two-tail
Vibrio cholerae (cfu/100 ml)	1216	16	0.21	0.28
Aeromonas (cfu/100 ml)	38180	23882	0.65	0.64
Coliforms (MPN per 100 ml)	1106	679	0.23	0.11
E. coli (MPN per 100 ml)	3	5	0.21	0.34
HPCs (cfu/ml)	79220	1120	0.07	0.02