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Occurrence of *Aeromonas hydrophila* in Southern California's Coastal Waters and Virulence Factors Associated with Infections

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Occurrence of *Aeromonas hydrophila* in Southern California's Coastal Waters and Virulence  
Factors Associated with Infections

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*Aeromonas hydrophila*, a ubiquitous aquatic microorganism, is an opportunistic pathogen that has been associated to wound infections, gastroenteritis, septicemia, and traveler's diarrhea in humans and hemorrhagic septicemia in fish. The main routes of exposure in humans are ingestion of contaminated foods and drinking water, or direct contact with recreational waters. Human exposure to *Aeromonas* has risen due to the increased usage of aquatic recreational sites especially in Southern California with its warm climates throughout most of the year, attracting people to its beaches. The pathogenicity of aeromonads has been linked to exotoxins such as cytolytic enterotoxin, hemolysin/aerolysin, lipases, and proteases. While the majority of superficial wound infections have been mild and self-limiting, there has been an increase in the number of *A. hydrophila* associated disease. Furthermore, serious tissue damage requiring hospitalization and aggressive anti-microbial therapy have been reported in healthy individuals, who were infected with virulent *A. hydrophila* from environmental waters. *A. hydrophila* was listed on EPA's Drinking Water Contaminant Candidate List (1998), and there are concerns that traditional bacterial indicators may not be sufficient identifiers of potential waterborne pathogens (NRC, 2004; Dumontet et al, 2000; Araujo et al, 1990; Rippey & Cabelli, 1980).

This study examined *Aeromonas* levels and associated virulence genes (cytolytic enterotoxin/ hemolysin and serine protease) in Southern California's major river mouths and the surf zones of associated beaches to understand the potential health risk from *Aeromonas* infections. *Aeromonas* spp. were present in every monthly or quarterly water samples from the seven river mouths and six associated beaches in the four counties (LA, Orange, San Diego, and Ventura) tested. The levels ranged from  $10^1$  to  $10^{10}$  cfu (colony forming unit) / 100 ml from March 2001 to March 2002. However, the ranged contracted to concentrations of  $10^1$  to  $10^5$  cfu/ 100 ml when the one storm event was excluded from the data. These levels were comparable to the *Aeromonas* levels in the European studies (Araujo et al, 1990; Dumontet et al, 2000; Fiorentini et al, 1998), but were lower than the US study on the Anacostia River (Seidler et al, 1980). In this study, the levels of aeromonads reported were not statistically different ( $p > 0.05$ ) within and between river mouths and surf zones in each county, using a Kruskal-Wallis One way Analysis of Variance. And although some studies have found seasonal cycles, the lack of seasonal significance in this study is consistent with other studies (Fiorentini et al, 1998; Mateos et al, 1992; Araujo et al, 1990).

In the LA and Orange Counties, the levels peaked in the summer ( $8.61 \times 10^3$  cfu/ 100ml) and in the fall ( $5.04 \times 10^4$  cfu/ 100ml) based on the geometric mean seasonal data, excluding and including storm events, respectively. The one storm event that was captured showed several orders of magnitude higher of *Aeromonas* counts. This could be due to bacterial input from soil or other terrestrial sources and/or nutrient input from runoff, which could contributed to the growth of *Aeromonas* spp. Further sampling during a storm event would be required to determine if the increase in *Aeromonas* levels are recurring events and whether a statistical difference between levels in non-storm and storm events is present.

In this study, pH ranged from 3.4 to 8.6 with the majority of values falling between pH 7.5 and 8.6 for samples taken in all four counties. Temperature oscillated from 13° to 28°C while salinity varied from 2 to 36 ppt. The fluctuations of these three physical/chemical conditions were well within the lower and upper limits for *Aeromonas* growth. Samples in LA and Orange Counties were taken below 1.6 ft-tidal levels. In San Diego and Ventura Counties, samples were taken at low to mid and low to high tides, respectively. No correlations were demonstrated between physical or chemical conditions such as pH, temperature, salinity, or tide levels and colony counts, which were consistent with some of the literature (Maalej et al, 2003; Sautour et al, 2003; Fiorentini et al, 1998; McClure et al, 1994; Rippey & Cabelli, 1980).

The pathogenicity of *A. hydrophila* has been associated with virulence factors such as hemolysins, cytolytic enterotoxin, elastase, and lipases. The enterotoxin (hemolysins and cytolytic enterotoxin) has been widely regarded a major constituent in *A. hydrophila* associated infections. The enterotoxin is secreted as inactive toxin and can be activated by the serine protease. The presence of virulence factors might be a better indicator of the potential health risk to bathers than counts alone. Therefore in this study, we determined the frequency of these virulence factors among the *Aeromonas* coastal water populations.

Using a 3-Tube Most Probable Number Method coupled with a single PCR assay, 71-100% and 84-100% of the samples contained the toxin and the serine protease activator genes, respectively. For the five sites spanning LA and Orange Counties, 58 out of 63 (92%) of the samples were positive for the toxin gene while only 53 out of 63 (84%) the samples were spg positive. In San Diego, 10 out of 14 (71%) and 12 out of 14 (86%) of the samples were PCR positive for the toxin gene and spg, respectively. In Ventura County, all samples (100%) from the four sites were positive for the toxin and activator genes.

The prevalence of the toxin gene ranged from 1 cell with the trait : 1 *Aeromonas* viable counts per sample to 1 : 1 x 10<sup>8</sup>. The prevalence of the serine protease gene was lower than the enterotoxin gene and ranged from 1 : 1 to 1 : 2.6 x 10<sup>8</sup>. Although colony counts showed no geographical or seasonal statistical differences among sites within the four counties, the highest geometric mean prevalences of the enterotoxin gene occurred during summer and winter in the majority of the non-storm event samples.

In the surf zones of LA and Orange County beaches, a high prevalence (1:4 to 1:9) of the toxin gene occurred in 32% of the coastal samples, which suggested a potential health risk for bathers. Furthermore, 44% of the samples showed the occurrence of the enterotoxin gene at a frequency of 20-100% of the *Aeromonas* environmental water populations. Also, there was no temporal difference in toxin occurrence within the *Aeromonas* populations over the 12-13 months of sampling and the persistence of these virulent populations suggested a potential health risk throughout the year.

In San Diego, the peak frequency of the toxin gene occurred in winter season (1:1 to 1:9). Although no fall sample was available for Ventura County sites, winter again represented the highest prevalence (1:2 to 1:7) in the seasons tested. Furthermore, 50% of the samples in these two counties have the toxin gene frequency of 20-100% in the *Aeromonas* water populations.

In the second year of this project, an increase in detection method was developed with the availability of a quantitative PCR (qPCR) machine. Samples that were negative by single PCR were subjected to qPCR with a dual labeled probe. The detection limit ranged from 1-10 copies of the toxin gene. Negative samples by single PCR were positive by qPCR. A paper based on this finding was published in the Coastal Environment 2004: Fifth International Conference on Environmental Problems in Coastal Regions (Wessex Institute of Technology).

Furthermore, 11-fold increase in detection sensitivity was observed using qPCR. There was statistically significant difference in the prevalence of the toxin gene between the two methods. Besides increasing the detection sensitivity, QPCR had faster analysis time and direct toxin copy number was measured. The geometric mean of the copy number was 10 copies of the toxin gene per cell, based on 27 samples (31 dilutions). The prevalence was 1 (cell contained the hemolysin gene) : 10 (*Aeromonas* spp.) by copy number calculations.

However, it should be cautioned that the expression of virulence factors should be ascertained in virulent *A. hydrophila*. Although the high prevalence of the toxin gene is of concern, the question of whether the toxin is produced when the organism enters a host is still unknown. The conditions which facilitate toxin production are currently being explored by other researchers as well as this lab. The findings made by this funding have lead to the current experiments on the expression of the hemolysin toxin of *A. hydrophila* isolated from marine water. A better understanding of the pathogenic mechanism may shed light as to the potential health risk of virulent *A. hydrophila*.

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