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Effects of Chemical Parameters of Water in the Prevalence of *Vibrio cholerae* O1 and O139

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Abstract:
A study under the caption 'Effects of chemical parameters of water in the prevalence of Vibrio cholerae O1 and O139' was conducted to investigate how different chemical parameters of water affect the abundance of these two pathogenic bacteria. The metadata analysis as per bivariate analysis has revealed that salinity, temperature and pH have significant correlation with VCO1 and VCO139 in nearly all the studied sites but dissolved oxygen (DO) and conductivity have less or no influence in the fluctuations of these two pathogenic bacteria. Canonical Correspondence Analyses (CCS) regarding multivariate analysis has revealed that VCO139 has much stronger correlation with salinity, pH and temperature than that of VCO1.

Keywords*: Vibrio cholera (VC), pH, DO, conductivity, salinity*

1. Introduction

Cholera is endemic in the Ganges delta, occurring twice yearly in epidemic form (Alam *et. al.,* 2006). It is also a major health problem for countries of Africa, Latin America and Asia and *V. cholerae* O1 is native to both marine and freshwater environments where it exists in association with planktons (Albert *et. al.,* 1993). Abundance of toxigenic *Vibrio cholerae* has been found to be correlated with chemical parameters of water (Singleton *et. al*., 1982). Collwell *et. al.*(1990) reported that the growth of *Vibrio cholerae* O1 is influenced by salinity of 2 to 20 ppt and water temperature less than 17^{o}C. Several environmental parameters of the seasonal cycle of cholera in India and Bangladesh where cholera is endemic have been identified (Baumann *et. al.*, 1984). Ocean surveillance by satellite remote sensing was used to monitor changes in sea surface temperature (SST) and sea surface height (SSH) in the Bay of Bengal. The pattern of changes in these parameters were shown to be related to *V. cholerae* dynamics in coastal, estuarine, and riparian waters of the Bay of Bengal and to the cholera epidemics caused by these bacteria in that region of the world (Lobitz *et. al.*, 1994). *V. cholerae* requires 5 to 15 ppt NaCl for optimum growth but will usually grow in complex medium without added salt (nutrient broth, for example), although growth is reduced to 50 % to 80% of optimal growth (Reichelt and Baumann, 1974).

Effect of chemical water parameters in the survival and pathogenicity of *V. cholarae* O1 and O139 in Bangladesh perspective is scarce, whereas there are sporadic, epidemic and seasonal cholera outbreaks each year across the length and breadth of the country which might be influenced by the diverse limnological parameters. In the backdrop of such predicament, the current study was conducted to add some light to the debate regarding how the ecology of *Vibrio cholera* is affected by pH, temperature, salinity, conductivity and dissolved oxygen (DO) in the south western coastal waters of Bangladesh.

2. Materials and Methods

Sampling was carried out for a prolonged period of 36 months during the period from October 2007 to September 2010 (October to January, February to May and June to September were treated as winter, summer and monsoon respectively for convenience). The present study was conducted at three selected ponds of Mathbaria, which is geographically adjacent to the coast of the Bay of Bengal and approximately 400 km southwest of Dhaka. The geographical location of the study area was between 22° 29' N to 90°-22' E. In each round, a 5 liter sampling bottle was filled with water for 20 times from different areas of each pond and accordingly 100 liter water was filtered through 64-µm and 20-µm-pore-sized nylon nets (Millipore Corp., Bedford, and Mass). The 64-µm-pore-sized net was placed sequentially in front of the 20-µm mesh-sized nylon net, with each having a collecting bucket at the base of the net. In this way, 100 litres of water was filtered from each pond in each round in order to get the final concentration of 50 ml separately from two nets with a view to analyzing phyto- and zooplanktons respectively along with their possible

attachment with *Vibrio cholerae*. The nets were hung high while the plankton-free water was filtering out from where 200 ml water sample was collected into another bottle. From each of 50 ml samples, 10 ml was transferred into another small vial along with required preservative (formalin for zooplankton and Lugol's iodine for phytoplankton). So, there were three types of samples, viz, 10 ml phytoplankton sample, 10 ml zooplankton sample and 200 ml plankton-free water sample in separately labelled vials for each pond and accordingly 9 types of samples were collected from three ponds in each turn. The same trend of sample collection was continued for consecutive 36 months totalling the annual number of samples as 108 and sum total of samples for three consecutive years were 324 (108 water samples, 108 zooplankton sample and 108 phytoplankton samples). All samples were collected in sterile dark Nalgene bottles (Nalgene Nunc International, St. Louis, Mo.) by following aseptic technique along with appropriate preservatives for phyto- and zooplanktons, placed in an insulated plastic box and transported at ambient air temperature from the site of collection to the central laboratory of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), in Dhaka. Both the samples from 64-µm and 20-µm mesh sized nets were further concentrated in the laboratory to a final volume of 5 ml by filtering through a 0.22-µm-pore-size bacteriological membrane filter (Millipore) and the retained contents on the membrane filter were washed into phosphate-buffered saline (pH 8.0). Limnological parameters like pH, dissolved oxygen (DO), temperature, salinity and conductivity were recorded *in situ* using relevant digital devices (Portable Multiparameter Meter, HACH Company, Loveland, Colorado 80539-0389).

Study area

From 50 ml, 10 ml was for analysis and the samples were immediately preserved by 5% buffered formaldehyde. For qualitative and quantitative study, samples were observed under a compound microscope in a S-R (Sedgwick-Rafter) cell. Before filling the S-R cell with sample the cover slip was placed diagonally across the cell. The sample was transferred with a large pipette. Placing the cover slip in this manner is to prevent formation of air bubbles. Then cover slip was rotated to cover the inner portion and then count was made under microscope.

3. Enrichment, Plating and Biochemical test

Samples were enriched in alkaline peptone water referred as APW (Difco, Detroit, MI) and incubated at 37°C for 6 to 8 hours before plating on TCBS agar (Eiken, Tokyo, Japan) and TTGA (Difco). APW contained 1% peptone and 1% sodium chloride with the pH 8.5. Approximately, 5 µL of enriched APW broth was streaked by using an inoculating loop on both thiosulfate-citrate-bile salts-sucrose (TCBS), and taurocholate-tellurite-gelatin agar (TTGA) and incubated at 37°C for 18 to 24 hours. Colonies with the characteristic appearance of *Vibrio cholerae* were confirmed by biochemical tests like KIA (Kligler's iron agar), TSI (triple sugar iron agar, oxidase, gas production from glucose, sucrose, lysine, arginine, ornithine, VP (Voges-Proskaeur) etc. Finally, serological tests were done using polyvalent and monoclonal antibodies specific for *V. cholerae* O1 and O139. Samples were preincubated overnight, in the dark, with 0.025% yeast extract (Difco) and 0.002% nalidixic acid (Sigma-Aldrich, St. Louis, MO). The samples were then centrifuged and the pellet was stained with cholera DFA reagents like fluoresce in isothio cyanate-labelled antiserum specific for O1 or O139 (New Horizon Diagnostics, Columbia, MD). Fluorescent stained cells were observed and counted under UV light by using an epifluorescence microscope (Olympus Bx51) and recorded with the help of a digital camera attached with the same microscope (Olympus DP20).

4. Direct Fluorescent Antibody (DFA) Procedure

A thin smear was made by adding 5 µL of re-suspended sample in a well on a slide and the contents were spread to cover the well. A control thin smear was made without samples (negative control). They were dried in air or incubated at 37ºC for drying. Then 5 µL of absolute methanol or ethanol was added to each control or sample well to fix the smear and then air dried and 10 µL of reconstituted cholera DFA reagent was added to each well. The slides were placed in a covered, moist chamber and incubated at 37ºc for 30 ± 5 minutes. The slides were thoroughly rinsed with phosphate buffer solution (PBS). Excess moisture was absorbed using a blotting paper. A drop of fluorescent mounting medium was added on the slide and then it was covered with a 22 × 50 mm cover-slip. The slides were viewed immediately at a magnification of 1000 X with oil immersion.

5. Statistical Analysis

Pearson correlation and canonical correspondence analyses (CCA) were used to investigate the associations of VCO1 and VCO139 with pH, salinity, conductivity, dissolved oxygen and temperature. Pearson correlation coefficients were computed for the dominant species using SPSS® 16.0 (© SPSS Inc., Chicago, IL. 2007) to study the correlations between *Vibrio cholera*e O1 and O139 abundance and water parameters. Canonical correspondence analyses (CCA) have been done using CANOCO 4 for Windows to further explore the associations between species abundance with chemical parameters.

6. Results and Discussion

Both bivariate and multivariate analyses in Table-1 and Table-2 revealed that the prevalence of VCO1 is strongly correlated with the average pH at 0.01% level only in site-1 and fairly correlated at 0.05% level in site-3. However site-2 showed insignificant correlation. The same pattern of significant correlation is noticeable between VCO1 and salinity. DO has not been found to be correlated with the VCO1 as well as VCO139. Strong correlation is found between VCO1 and conductivity only in site-3 at 0.01% significant level and at 0.05% level in site-1 but no such correlation is found in site-2. Temperature has been found to be strongly correlated with the findings of VCO1 at 0.01% significant level in site-1 and site-3 but no correlation has been noticed in site-2. Regarding VCO139 the pH, salinity and temperature have been found to be strongly correlated at 0.01% significant level in all sites. Conductivity showed strong positive correlation at 0.01% significant level only in site-3 but in site-1 and site-2, no correlation has been noticed.

	Variable	**VCO1**		**VCO139**	
		Pearson coefficient (r)	**P value**	**Pearson coefficient (r)**	**P value**
SITE 1	Ph	.844**	.004	.977**	.000
	Salainity	.831**	.005	.978**	.000
	DO	-.256	.507	.116	.767
	Conductivity	.734*	.024	.444	.231
	Temperature	.907**	.001	.992**	.000
SITE 2	pH	.555	.121	.962**	.000
	Salainity	.582	.100	.980**	.000
	DO	.142	.716	.538	.136
	Conductivity	.197	.612	.423	.256
	Temperature	.583	.100	.960**	.000
SITE3	pH	.728*	.026	.911**	.001
	Salainity	.791*	.011	.945**	.000
	DO	-.316	.407	-.388	.302
	Conductivity	-.874**	.002	-.667*	.050
	Temperature	.971**	.000	.983**	.000

Table 1: Pearson correlation analysis (Bivariate)

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

	Variable	**Lambda**	**F value**	**P value**
SITE1	Temperature	0.90	66.46	0.002
	conductivity	0.09	41.75	0.002
	salainity	0.00	1.69	0.256
	DO	0.00	3.14	0.162
	pH	0.01	0.11	0.786
SITE2	Temperature	0.01	0.08	0.800
	conductivity	0.00	0.03	0.868
	salainity	0.35	3.80	0.084
	DO	0.06	0.60	0.490
	pH	0.02	0.12	0.742
SITE3	Temperature	0.95	125.62	0.002
	conductivity	0.04	16.87	0.002
	salainity	0.00	3.10	0.130
	DO	0.01	5.50	0.066
	pH	0.00	0.17	0.782

Table 2: Multivariate analysis

In site-1, canonical correspondence analysis (CCS) in Fig-1 shows that the findings of VCO139 is strongly correlated with salinity, pH and temperature than that of VCO1. Average DO and conductivity have no such correlation with the findings of both VCO1 and VCO139. Fig-2 shows that in site-2, VCO139 has been found to be strongly correlated with temperature, salinity and

pH than that of VCO1. However, DO and conductivity show no positive correlation. In site-3 both VCO1 and VCO139 is correlated with pH, salinity and temperature but conductivity and DO show no correlation (Fig-3).

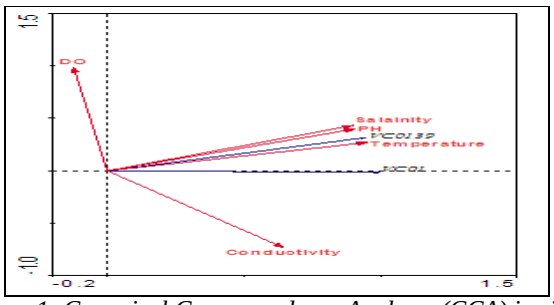
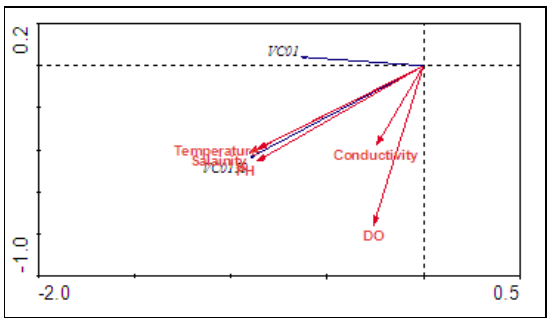


Figure 1: Canonical Correspondence Analyses (CCA) in site 1



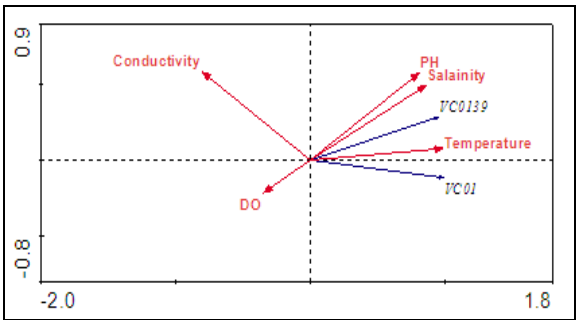


Figure 2: Canonical Correspondence Analyses (CCA) in site 2
Figure 3: Canonical Correspondence Analyses (CCA) in site 3

It was found in the present study that the optimum temperature for ideal growth of *V. cholerae* was ranged from 30°C to 33.75°C and highest count of VCO1 and VCO139 were reported in summer. Colwell *et. al.* (1981) also found that ideal growth temperature for VCO1 biotype El Tor varies between 30 and 37°C. The current study found that slightly alkaline pH of water favours the promotion of VCO1 and VCO139 and their growth has been found to be decreased significantly by the acidic pH. Mujica *et. al.* (1982) found that tomato sauce with pH 4.5 and 4.1 are sufficient to inactivate VCO1.

7. References

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