

Viability of *Vibrio cholerae* in Water

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The viability of *V. cholerae* Inaba and Ogawa in distilled water, municipal water and sea water both at room temperature (28-30°C) and at refrigerated temperatures (5-10°C) is discussed. The survival varied from 20 h in distilled water at room temperature to 38-40 days in sterile sea water at room temperature. Extended viability was noticed in all cases at refrigerated temperature and maximum viability of 165-170 days was noticed in sterile sea water at 5-10°C.

Transmission of *Vibrio cholerae* from person to person during cholera epidemic is reported to be through environmental factors like food, water, flies etc. Often seafoods have been found to be a source of *V. cholerae* during cholera outbreaks (Angelo De Paolo, 1981). Since water is used in large quantities during different stages of seafood processing, it can be a major source of contamination if *V. cholerae* is present in the water. It is known that this pathogen can survive in water for a long period (ICMSF, 1978). However, much work has not been done on the viability of *V. cholerae* in water except a few references by Prescott & Bhattacharjee (1969) and Barua (1970). So studies were taken up by the authors to determine the viability of *V. cholerae* in water collected from different sources and the results of the study are presented in this paper.

Materials and Methods

Distilled water, municipal water, sterile municipal water, sea water and sterile sea water were inoculated with pure strains of *V. cholerae* after 16-18 h growth in Alkaline Peptone Water (APW) at 37°C. Both EIT or Inaba and Ogawa cultures one each isolated from seafoods by the authors and later got confirmed at National Institute of Cholera and Enteric Diseases, Calcutta were used for the study. The water samples after inoculation were stored in glass containers at room temperature (28 to 30°C) and at refrigerated temperature (5 to 10°C). Samples (250 ml) were drawn periodically and were tested for the organism.

The sample was added to equal volume of double strength APW and incubated at 37°C for 16-18 h. Then one ml. from the surface was transferred to 9 ml APW for the second enrichment at 37°C for 6-9 h. From both the enrichments 3 mm loopful from the surface was streaked on to Thiosulphate Citrate Bile salt Sucrose (TCBS) Agar plates. After incubation at 37°C for 18-24 h the suspected colonies on TCBS agar plates were confirmed by biochemical and serological tests as recommended by FDA (1978). pH and sodium chloride were estimated as per the method given in APHA (1960) and an initial count of *V. cholerae* was estimated by direct plating on TCBS agar plate.

Results and Discussion

The pH and sodium chloride content of water from different sources are given in Table 1.

After inoculation, the water sample had an initial load of 5000 *V. cholerae*/ml.

The viability of *V. cholerae* Inaba and Ogawa in different sources of water during storage at room temperature and at refrigerated temperature are presented in Table 2.

It is seen from the Table that in distilled water, at room temperature, *V. cholerae* survives only for a maximum period of 20 h whereas at refrigerated temperature the survival period is extended upto 5 days. In the municipal water the organism survives for a period of 35-40 h at room temperature and at refrigerated temperature the survival period is extended upto 5-10

Table 1. *pH and NaCl contents of water samples*

	pH	NaCl%
Distilled water	3.63	0
Municipal water	7.10	0.001
Sea water	7.70	1.700

days. Sterilization of the municipal water before incubation extended the survival period upto 2-3 days and 10-16 days at room temperature and refrigerated temperature respectively. The organism is viable in sea water for 9-10 days at room temperature and 60-70 days at refrigerated temperature. Felsenfeld (1965) has also reported that *V. cholerae* survive very well in sea water. But when sterile sea water was

used, the organism was viable for 30-40 days at room temperature and 165-170 days at refrigerated temperature. The results in general indicate that when other microorganisms are absent *V. cholerae* survive in water for longer periods and the viability of the organism is also extended considerably when kept at refrigerated temperature. This is a fall agreement with the findings of Pollitzer (1959) and Felsenfeld (1974) who have stated that the survival of *V. cholerae* in water is dependent on temperature, pH, salt content, organic material and presents of competing bacteria.

The results in general, further indicate that *V. cholerae* Inaba has a slightly extended viability in most of the cases.

Table 2. *Viability of V. cholerae in different sources of water*

Source of water	El Tor Inaba		El Tor Ogawa	
	Room temperature	Refrigerated temperature	Room temperature	Refrigerated temperature
Distilled water	17-20 h	5 days	17 h	5 days
Municipal water	35-40 h	5.9 days	35-40 h	5-10 days
Sterile municipal water	2-3 days	10-16 days	2-3 days	10-16 days
Sea water	9-10 days	60-70 days	4-6 days	45-50 days
Sterile sea water	38-40 days	165-170 days	32-35 days	150-160 days

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